



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

| | | | |
|--|--|-----------|--|
| (51) International Patent Classification ⁶ : C12N 15/63, C07K 14/47, 14/515, C12N 15/62, 5/16, C07K 16/28 | | A1 | (11) International Publication Number: WO 95/13387 (43) International Publication Date: 18 May 1995 (18.05.95) |
| (21) International Application Number: PCT/EP94/03767 (22) International Filing Date: 12 November 1994 (12.11.94) (30) Priority Data: 08/152,552 12 November 1993 (12.11.93) US (71) Applicant: MAX-PLANCK-GESELLSCHAFT ZUR FÖRDERUNG DER WISSENSCHAFTEN E.V. [DE/DE]; Busenstrasse 10, D-37073 Göttingen (DE). (72) Inventor: RISAU, Werner; Dresdenerstrasse 2, D-35510 Butzbach (DE). (74) Agents: RUFF, Michael et al.; Willy-Brandt-Strasse 28, D-70173 Stuttgart (DE). | | | (81) Designated States: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SI, SK, TJ, TT, UA, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i> |
| (54) Title: TIE-2, A NOVEL RECEPTOR TYROSINE KINASE | | | |
| (57) Abstract The present invention relates to a novel receptor tyrosine kinase, herein referred to as <i>tie-2</i> , to nucleotide sequences and expression vectors encoding <i>tie-2</i> , and to methods of inhibiting <i>tie-2</i> activity. The invention also relates to other members of the <i>tie-2</i> receptor tyrosine kinase family. Genetically engineered host cells that express <i>tie-2</i> may be used to evaluate and screen drugs involved in <i>tie-2</i> activation and regulation. The invention relates to the use of such drugs as agonists or antagonists of <i>tie-2</i> activity. | | | |

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

| | | | | | |
|----|--------------------------|----|--|----|--------------------------|
| AT | Austria | GB | United Kingdom | MR | Mauritania |
| AU | Australia | GE | Georgia | MW | Malawi |
| BB | Barbados | GN | Guinea | NE | Niger |
| BE | Belgium | GR | Greece | NL | Netherlands |
| BF | Burkina Faso | HU | Hungary | NO | Norway |
| BG | Bulgaria | IE | Ireland | NZ | New Zealand |
| BJ | Benin | IT | Italy | PL | Poland |
| BR | Brazil | JP | Japan | PT | Portugal |
| BY | Belarus | KE | Kenya | RO | Romania |
| CA | Canada | KG | Kyrgyzstan | RU | Russian Federation |
| CF | Central African Republic | KP | Democratic People's Republic of Korea | SD | Sudan |
| CG | Congo | KR | Republic of Korea | SE | Sweden |
| CH | Switzerland | KZ | Kazakhstan | SI | Slovenia |
| CI | Côte d'Ivoire | LI | Liechtenstein | SK | Slovakia |
| CM | Cameroon | LK | Sri Lanka | SN | Senegal |
| CN | China | LU | Luxembourg | TD | Chad |
| CS | Czechoslovakia | LV | Latvia | TG | Togo |
| CZ | Czech Republic | MC | Monaco | TJ | Tajikistan |
| DE | Germany | MD | Republic of Moldova | TT | Trinidad and Tobago |
| DK | Denmark | MG | Madagascar | UA | Ukraine |
| ES | Spain | ML | Mali | US | United States of America |
| FI | Finland | MN | Mongolia | UZ | Uzbekistan |
| FR | France | | | VN | Viet Nam |
| GA | Gabon | | | | |

TIE-2, A NOVEL RECEPTOR TYROSINE KINASE1. INTRODUCTION

The present invention relates to a novel receptor
5 tyrosine kinase, herein referred to as *tie-2*, to
nucleotide sequences and expression vectors encoding
tie-2, and to methods of inhibiting *tie-2* activity.
The invention also relates to other members of the
tie-2 receptor tyrosine kinase family. Genetically
10 engineered host cells that express *tie-2* may be used
to evaluate and screen drugs involved in *tie-2*
activation and regulation. The invention relates to
the use of such drugs as agonists or antagonists of
tie-2 activity.

15

2. BACKGROUND

Receptor tyrosine kinases comprise a large and
evolutionarily conserved family of proteins comprised
of an extracellular ligand-binding domain, a
20 transmembrane domain and an intracellular tyrosine
kinase domain. Receptor tyrosine kinases are involved
in a variety of critical cellular processes such as
cellular differentiation and proliferation. The
binding of ligand to receptor tyrosine kinases induces
25 the formation of receptor dimers followed by
activation of receptor tyrosine kinase activity. This
in turn results in phosphorylation of a number of
intracellular substrates leading to a cascade of
events eventually resulting in cellular responses such
30 as changes in gene expression, cell morphology and
cell proliferation.

The formation and spreading of blood vessels, or
vasculogenesis and angiogenesis, respectively, play an
important role in a variety of physiological processes
35 such as embryonic development, wound healing and organ

regeneration. In particular, endothelial cell proliferation plays an important role during the development of the vascular system. In *in vitro* systems of angiogenesis, molecules that regulate tyrosine kinase activity have been found to modulate the invasion and tube formation of endothelial cells indicating that tyrosine phosphorylation plays a critical role in the regulation of endothelial cell proliferation and morphogenesis.

The cloning and characterization of two novel tyrosine kinases, expressed in endothelial cells, has recently been reported in the literature. Partanean et al. (1992, Mol. Cell. Biol. 12:1698-1707) identified a receptor tyrosine kinase, referred to as *tie*, which has 76% amino acid sequence homology with *tie-2* in the cytoplasmic domain. The sequence homology between the two proteins diverges and is less pronounced in the extracellular and transmembrane domain (33% and 37% respectively). In addition, Dumont et al. (1992, Oncogene 7:1471-1480) reported the cloning and characterization of a partial cDNA clone encoding only the cytoplasmic domain of a novel receptor tyrosine kinase. The cloning of a full length representative of this particular clone, referred to as *tek*, has recently been reported and the deduced amino acid sequence of the *tek* tyrosine kinase receptor indicates broad homology with *tie-2* (Ziegler et al., 1993, Oncogene 8:663-670).

The identification of novel tyrosine kinase receptors mediating physiological processes such as vasculogenesis and angiogenesis will lead to a more complete understanding of the molecular mechanisms controlling blood vessel formation. In addition, the identification of novel receptors involved in these processes will provide targets for development of therapeutic applications designed to treat disorders associated with aberrant blood vessel formation.

3. SUMMARY OF THE INVENTION

The present invention relates to a novel receptor tyrosine kinase receptor, herein referred to as *tie-2*, to nucleotide sequences and expression vectors encoding *tie-2*, and to methods of inhibiting *tie-2* activity. The invention is based, in part, on the
5 isolation of a cDNA clone, from a brain capillary cDNA library, encoding the *tie-2* receptor tyrosine kinase.

The invention also relates to novel members of the *tie-2* family of receptor tyrosine kinases. More specifically, the invention relates to members of the
10 *tie-2* family of receptor tyrosine kinases that are defined, herein, as those receptors demonstrating 80% homology at the amino acid level in substantial stretches of DNA sequence with *tie-2*. In addition,
15 members of the *tie-2* family of receptor tyrosine kinases are defined as those receptors containing an intracellular tyrosine kinase domain and, in the extracellular region of the protein, EGF-like repeats flanked by a single amino terminal immunoglobulin (Ig)
20 domain and a carboxyl-terminal triplet of fibronectin (Fn) type III domains.

Northern blot analysis and in situ hybridization indicates that *tie-2* is expressed in endothelial cell precursors (angioblasts) and in endothelial cells of
25 the sprouting blood vessels throughout development and in all organs and tissues so far examined. Pharmaceutical reagents designed to modulate *tie-2* activity may be useful for treating diseases and/or processes associated with angiogenesis and
30 vasculogenesis.

The invention relates to expression systems designed to produce *tie-2* receptor and/or cell lines expressing *tie-2* receptor. For example, engineered
cell lines expressing *tie-2* on their surface may be
35 advantageously used to identify *tie-2* ligands and to screen and identify agonist and antagonist of the *tie-*

2 receptor. Additionally, expression of soluble recombinant *tie-2* may be used to generate antibodies against specific epitopes of the *tie-2* protein and/or to screen peptide libraries for molecules that bind *tie-2*.

5

4. BRIEF DESCRIPTION OF THE FIGURES

Fig. 1A, 1B and 1C. Nucleotide Sequence of murine *tie-2*. The AUG START codon and AGA STOP codon are underlined.

10

Fig. 2A. Deduced amino acid sequence and structure of *tie-2*. Amino acid sequence of *tie-2* in single letter code. The potential signal sequence cleavage site is indicated by an arrowhead. Black dots mark the two cysteine residues, possible involved in sulfhydryl bonding of the immunoglobulin domain. The three EGF-like repeats are boxed. The three fibronectin type III domains are underlined. The transmembrane region is given in bold face letters. The tyrosine kinase domain is indicated by shaded boxes. The RGD triplet is marked by an asterisk.

20

Fig. 2B. Schematic diagram of the structure of *tie-2*. Ig, immunoglobulin domain; EGF, EGF-like repeats; FN, fibronectin type III domains; Kinase, tyrosine kinase domain; KI, kinase insertion.

25

Fig. 3. Amino acid sequence comparison of *tek*, *TEK* and *tie* with *tie-2*. Amino acid residues identical to *tie-2* are represented by (-). Gaps are indicated by (.).

30

Fig. 4. Northern analysis of *tie-2* expression in brain capillaries and total brain tissue from postnatal day 4 (P4) mice. A single transcript of approximately 4.7 kb, highly enriched in the capillary fraction was detected.

35

Fig. 5. *tie-2* expression during brain development. Sagittal sections of adult brain (A,B), postnatal day 4 brain (C,D,G,H) and embryonic day 12.5

brain (E,F) were hybridized with a *tie-2* probe. Bright-field (A,C,E) and corresponding dark-field micrographs (B,D,F) Arrowheads indicate expression in capillaries and arrows indicate hybridization signals over meningeal blood vessels. Higher magnification of a capillary sprout at the dorsal surface of a postnatal day 4 telencephalon (G). Higher magnification of a capillary in a deeper layer of the brain (H). No other than the vascular elements of the brain were found to synthesize *tie-2* mRNA. LM, leptomeninges; LV, lateral ventricle; TE, telencephalon; (A,B,C,D,E,F) bar represents 110 μm ; (G,H) bar represents 10 μm ;

Fig. 6. Colocalization of *tie-2* expression with immunostaining for PECAM (CD31). Adjacent section of E12.5 embryos were hybridize with a *tie-2* probe (A) or stained with antibody recognizing PECAM (CD31)(B). Arrowheads indicate expression of *tie-2* in the endothelial cell layer of a medium-sized blood vessel from the head region. Bar represents 10 μm .

Fig. 7. *Tie-2* expression in organs and tissues of E 12.5 embryos and colocalization with immunostaining for PECAM (CD31). Adjacent sections of E 12.5 embryos were stained with an antibody recognizing PECAM (CD31) (A,C,E,G) or hybridized with a *tie-2* probe (B,D,F,H). The expression pattern of *tie-2* was found to be identical to the staining pattern fort PECAM (CD31). TE, telencephalon; LV, lateral ventricle; CP, choroid plexus of the lateral ventricle; DA, dorsal aorta; SC, spinal cord; EN, endocardium; MY, myocardium; BR, bronchus; SO, somite; Bar represents 110 μm .

Fig. 8. *Tie-2* expression at embryonic day 8.5. Transverse section of an E 8.5 embryo and adjacent yolk sac. Hybridization signals were detectable in the endocardium, dorsal aorta, cardinal vein and the mesodermal (inner layer) of the yolk sac (A,B).

Arrowheads indicate hybridization signal over the marginal cells of an advanced stage blood island (C,D). Note the absence of hybridization signals in the neuroectoderm. CV, cardinal vein (head vein); BI, blood island; DA, dorsal aorta; EN, endocardial tissue; TE, telencephalon; YS, yolk sac. Bar, represents 50 μ m.

5. DETAILED DESCRIPTION

The present invention relates to a novel receptor tyrosine kinases referred to herein as *tie-2*. The invention is based, in part, on the isolation of a cDNA clone encoding the *tie-2* receptor tyrosine kinase from a brain capillary cDNA library. The invention also relates to novel members of the *tie-2* receptor tyrosine kinase family as defined herein.

Results from Northern Blot analysis and *in situ* hybridization indicates that *tie-2* is expressed specifically in the endothelial cell lineage. *Tie-2* transcripts could be detected in endothelial cell precursors (angioblasts) and in endothelial cells of sprouting blood vessels throughout development and in all organs and tissues so far examined.

The invention relates to expression of the *tie-2* receptor and/or cell lines that express the *tie-2* receptor which may be used to screen for antibodies, peptides, or other ligands that act as agonists or antagonists of the *tie-2* receptor. For example, anti-*tie-2* antibodies may be used to inhibit *tie-2* function. Alternatively, screening of peptide libraries with recombinantly expressed soluble *tie-2* protein or cell lines expressing *tie-2* protein may be useful for identification of therapeutic molecules that function by regulating the biological activity of *tie-2*.

For clarity of discussion, the invention is described in the subsections below by way of example

for the murine *tie-2*. However, the principles may be analogously applied to clone and express the *tie-2* receptor of other species including humans.

5.1. THE TIE-2 CODING SEQUENCE

5 The nucleotide coding sequence and deduced amino acid sequence of the *tie-2* gene is depicted in Figures 1A, 1B, 1C (SEQ. ID NO: __) and Figure 2A (SEQ. ID NO: __), respectively. In accordance with the invention, any nucleotide sequence which encodes the amino acid sequence of the *tie-2* gene product can
10 be used to generate recombinant molecules which direct the expression of *tie-2*. In additional embodiments of the invention, nucleotide sequences which selectively hybridize to the *tie-2* nucleotide sequence shown in FIG. 1 (SEQ ID NO: __) may also be used to express
15 gene products with *tie-2* activity. Hereinafter all such variants of the *tie-2* nucleotide sequence will be referred to as the *tie-2* DNA sequence.

 In a specific embodiment described herein, the
20 *tie-2* gene was isolated by performing a polymerase chain reaction (PCR) in combination with two degenerate oligonucleotide primer pools that were designed from conserved protein regions of tyrosine kinases. As a template cDNA synthesized by reverse transcription of purified mRNA from capillaries of
25 pooled P4-P8 mice brains was used. Gel-purified reaction products of the expected size were radiolabelled and used directly to screen a cDNA library constructed from the remainder of the capillary mRNA. Sequence analysis of the inserts from
30 positive phages revealed that 13 of the cDNAs were derived from one mRNA species. The longest of these 13 clones, a fragment of 4640 bp, was sequenced completely. It contained a long open reading frame
35 encoding a protein of 1123 amino acid residues.

Within the extracellular part, the FASTA program detected homologies to proteins including TAN-1 (Ellisen et al., 1991 Cell 66:649-661), Xotch (Coffman et al., 1990), Laminin (Sasaki et al., 1988 J. Biol. Chem. 263:16536-16544). Delta (Vässin et al., 1987 EMBO J. 6:3431-3440) and Perlecan (Noonan et al., 1991 J. Biol. Chem. 266:22939-22947) (around 30% sequence identity). The structural basis for these homologies are three EGF-like repeats in the center of the extracellular portion of *tie-2* (Fig. 2A). EGF-like repeats consist of 30 to 40 amino acid residues often found in the extracellular parts of membrane-bound proteins or secreted proteins (Davies, 1990 New Biol 5:410-419). A common feature of these domains are six conserved cysteine residues, which are known to be involved in disulfide bonds. The three EGF domains in *tie-2* contain two additional cysteine residues at the carboxyl-terminal end of each repeat.

The central EGF-like repeats are flanked by two different structural motifs, a single amino-terminal immunoglobulin (Ig) domain and a carboxyl-terminal triplet of fibronectin (FN) type III domains. Although the Ig domain does not exhibit clear sequence similarities to other known proteins (except for *tie* and *TEK*), the Ig domain has the conserved features of a C2-set domain including typical residues surrounding the first cysteine residue and the canonical GxYxC found at the second cysteine residue (Williams and Barclay, 1988 Ann. Rev. Immunol. 6:381-405).

The three fibronectin type III (FN III) domains are most closely related to FN III repeats present in the protein-tyrosine phosphatases Delta (Krueger et al., 1990 EMBO J. 9:3241-3252), LAR (Streuli et al., 1988 J. Exp. Med. 168:1553-1562) and DLAR (Streuli et al., 1989 Proc. Natl. Acad. Sci USA 86:8698-8702) and to FN III repeats of the axonal glycoprotein TAG-1 (Furley et al., 1990 Cell 61:157-170) (around 20%

sequence identity). These domains have been described as units of approximately 90 amino acids containing hydrophobic residues at characteristic positions. In addition, a total of nine potential N-glycosylation sites are located in the extracellular part of tie-2.

The invention also relates to tie-2 genes isolated from other species, including humans. Members of the tie-2 family are defined herein as those receptors containing an intracellular tyrosine kinase domain, and, in the extracellular domain, EGF-like repeats flanked by two different structural motifs, a single amino-terminal immunoglobulin (Ig) domain and a carboxyl-terminal triplet of fibronectin (FN) type III domains. Such receptors may demonstrate about 80% homology at the nucleotide level, and even 90% homology at the amino acid level in substantial stretches of DNA sequence.

To isolate the tie-2 gene from other species a bacteriophage cDNA library may be screened, under conditions of reduced stringency, using a radioactively labeled fragment of the murine tie-2 clone. Alternatively the murine tie-2 sequence can be used to design degenerate or fully degenerate oligonucleotide probes which can be used as PCR probes or to screen bacteriophage cDNA libraries. A polymerase chain reaction (PCR) based strategy may be used to clone, for example, the human tie-2. Two pools of degenerate oligonucleotides, corresponding to conserved motifs within the murine tie-2 may be designed to serve as primers in a PCR reaction. Conserved motifs may include the tyrosine kinase domain, the EGF-like repeats, the immunoglobulin (Ig) domain or the fibronectin (Fn) type III domains. The template for the reaction is cDNA obtained by reverse transcription of mRNA prepared from cell lines or tissue known to express human tie-2 such as blood capillaries or endothelial cells. The PCR product may

be subcloned and sequenced to insure that the amplified sequences represent the *tie-2* sequences. The PCR fragment may be used to isolate a full length *tie-2* cDNA clone by radioactively labeling the amplified fragment and screening a bacteriophage cDNA library. Alternatively, the labeled fragment may be used to screen a genomic library. For a review of cloning strategies which may be used, see e.g., Maniatis, 1989, Molecular Cloning, A Laboratory Manual, Cold Springs Harbor Press, N.Y.; and Ausubel et al., 1989, Current Protocols in Molecular Biology, (Green Publishing Associates and Wiley Interscience, N.Y.)

Northern Blot analysis and *in situ* hybridization indicated that *tie-2* was expressed in blood vessels during brain angiogenesis. Figure 4 demonstrates the presence of a 4.7 Mb mRNA highly enriched in the capillary fraction. A single RNA species could also be detected in organs like the brain, kidney and heart.

In situ hybridization experiments indicated that *tie-2* mRNA was exclusively synthesized in the vasculature of adult brain, P4 brain, E12.5 brain E8.5 neuroectoderm (FIG. 5A-H). In P4 brain, *tie-2* expression was detected in capillaries that are about to invade the neural tissue as well as in vessels that have already reached deep layers of neuroectoderm. In addition to capillaries, meningeal blood vessels and the choroid plexus were also found to synthesize *tie-2* mRNA at comparable levels (FIGS. 5, 7B). At E12.5 the overall expression pattern was virtually identical to that observed at P4, although the density of labelled structures in E12.5 brain was reduced (Fig. 5C,O). This observation correlates with the less extensive vascularization of the embryonic brain (Bär, 1980 Adv. Anat. Embryol Cell Biol. 59:1-62). No hybridization signals were observed in the neuroectoderm of E8.5

embryos, because at that stage this tissue is still avascular (Fig. 8). In the adult brain, tie-2 expression was detectable, although it seemed to be reduced when compared to postnatal or embryonic stages. Hybridization signals persisted over larger vessels especially those of the meninges (Fig. 5A,B).
5 The synthesis of tie-2 mRNA in smaller vessels and capillaries was barely detectable.

To identify the cell type, expressing tie-2 mRNA, *in situ* hybridization and immunohistochemistry was performed. The pattern of tie-2 expression was
10 compared with the staining of cells with a monoclonal antibody against the endothelial cell-specific adhesion molecule PECAM (CD31). Figure 6 demonstrates the overlap between cells stained by the PECAM
15 antibody and those cells labeling with a tie-2 probe. Figure 7 presents a survey on the coexpression of tie-2 and PECAM in several organs and tissues and it is clear from the results that tie-2 is coexpressed with PECAM in several organs and tissues.

The possible role of tie-2 in early stages of
20 vascular development was investigated by *in situ* hybridization studies with E8.5 sections. The probe detected tie-2 mRNA in the mesodermal component of the yolk sac (FIG. 8). This finding is of particular
25 interest, because it is in the mesodermal component of the yolk sac that the first signs of blood vessel development are evident. Clusters of mesenchymal cells form the so-called blood islands. At the margin of these aggregates cells, the so-called angioblasts,
30 adopt an endothelial-like phenotype, whereas in the center cells differentiate into embryonic hemoblasts. Fig. 8 demonstrates that the peripheral angioblasts synthesize high levels of tie-2 mRNA.

Within the embryo proper, expression was seen in
35 the anlagen of the vascular system, e.g. in the developing endocardium, the dorsal aortae and the

cardinal veins (Fig. 8A,B). It is likely that these signals stem from intraembryonic endothelial cell precursors, which are present in these structures at that stage, *tie-2* expression could also be detected in the allantois and in blood vessels of the maternal decidua.

5

5.2. EXPRESSION OF *TIE-2* RECEPTOR AND GENERATION OF CELL LINES THAT EXPRESS *TIE-2*

In accordance with the invention, *tie-2*
10 nucleotide sequences which encode *tie-2*, peptide fragments of *tie-2*, *tie-2* fusion proteins or functional equivalents thereof may be used to generate recombinant DNA molecules that direct the expression of *tie-2* protein or a functionally equivalent thereof,
15 in appropriate host cells. Alternatively, nucleotide sequences which hybridize to portions of the *tie-2* sequence may also be used in nucleic acid hybridization assays, Southern and Northern blot analyses, etc.

20 Due to the inherent degeneracy of the genetic code, other DNA sequences which encode substantially the same or a functionally equivalent amino acid sequence, may be used in the practice of the invention for the cloning and expression of the *tie-2* protein.
25 Such DNA sequences include those which are capable of hybridizing to the murine *tie-2* sequence under selective conditions.

Altered DNA sequences which may be used in accordance with the invention include deletions, additions
30 or substitutions of different nucleotide residues resulting in a sequence that encodes the same or a functionally equivalent gene product. These alterations would in all likelihood be in regions of *tie-2* that do not constitute functionally conserved
35 regions. In contrast, alterations, such as deletions,

additions or substitutions of nucleotide residues in functionally conserved *tie-2* regions would be expected to result in a nonfunctional *tie-2* receptor. The gene product itself may contain deletions, additions or substitutions of amino acid residues within the *tie-2* sequence, which result in a silent change thus producing a functionally equivalent *tie-2*. Such amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipatic nature of the residues involved. For example, negatively charged amino acids include aspartic acid and glutamic acid; positively charged amino acids include lysine and arginine; amino acids with uncharged polar head groups having similar hydrophilicity values include the following: leucine, isoleucine, valine; glycine, alanine; asparagine, glutamine; serine, threonine; phenylalanine, tyrosine. The DNA sequences of the invention may be engineered in order to alter the *tie-2* coding sequence for a variety of ends including but not limited to alterations which modify processing and expression of the gene product. For example, mutations may be introduced using techniques which are well known in the art, e.g. site-directed mutagenesis, to insert new restriction sites, to alter glycosylation patterns, phosphorylation, etc. For example, in certain expression systems such as yeast, host cells may over glycosylate the gene product. When using such expression systems it may be preferable to alter the *tie-2* coding sequence to eliminate any N-linked glycosylation site.

In another embodiment of the invention, the *tie-2* or a modified *tie-2* sequence may be ligated to a heterologous sequence to encode a fusion protein. For example, for screening of peptide libraries it may be useful to encode a chimeric *tie-2* protein expressing a heterologous epitope that is recognized by a

commercially available antibody. A fusion protein may also be engineered to contain a cleavage site located between the *tie-2* sequence and the heterologous protein sequence, so that the *tie-2* may be cleaved away from the heterologous moiety.

5 In an alternate embodiment of the invention, the coding sequence of *tie-2* could be synthesized in whole or in part, using chemical methods well known in the art. See, for example, Caruthers, et al., 1980, Nuc. Acids Res. Symp. Ser. 7:215-233; Crea and Horn, 180, Nuc. Acids Res. 9(10):2331; Matteucci and Caruthers,
10 1980, Tetrahedron Letters 21:719; and Chow and Kempe, 1981, Nuc. Acids Res. 9(12):2807-2817. Alternatively, the protein itself could be produced using chemical methods to synthesize the *tie-2* amino acid sequence in whole or in part. For example, peptides can be
15 synthesized by solid phase techniques, cleaved from the resin, and purified by preparative high performance liquid chromatography. (e.g., see Creighton, 1983, Proteins Structures And Molecular Principles, W.H. Freeman and Co., N.Y. pp. 50-60). The composition of the synthetic peptides may be confirmed by amino acid analysis or sequencing (e.g., the Edman degradation procedure; see Creighton, 1983, Proteins, Structures and Molecular Principles, W.H. Freeman and
20 Co., N.Y., pp. 34-49.

25 In order to express a biologically active *tie-2*, the nucleotide sequence coding for *tie-2*, or a functional equivalent, is inserted into an appropriate expression vector, i.e., a vector which contains the necessary elements for the transcription and
30 translation of the inserted coding sequence. The *tie-2* gene products as well as host cells or cell lines transfected or transformed with recombinant *tie-2* expression vectors can be used for a variety of purposes. These include but are not limited to
35 generating antibodies (i.e., monoclonal or polyclonal)

that bind to the receptor, including those that competitively inhibit binding of *tie-2* ligand and "neutralize" activity of *tie-2* and the screening and selection of drugs that act via the *tie-2* receptor; etc.

5

5.2.1. EXPRESSION SYSTEMS

Methods which are well known to those skilled in the art can be used to construct expression vectors containing the *tie-2* coding sequence and appropriate transcriptional/translational control signals. These methods include in vitro recombinant DNA techniques, synthetic techniques and in vivo recombination/genetic recombination. See, for example, the techniques described in Maniatis et al., 1989, Molecular Cloning A Laboratory Manual, Cold Spring Harbor Laboratory, N.Y. and Ausubel et al., 1989, Current Protocols in Molecular Biology, Greene Publishing Associates and Wiley Interscience, N.Y.

A variety of host-expression vector systems may be utilized to express the *tie-2* coding sequence. These include but are not limited to microorganisms such as bacteria transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing the *tie-2* coding sequence; yeast transformed with recombinant yeast expression vectors containing the *tie-2* coding sequence; insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus) containing the *tie-2* coding sequence; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing the *tie-2* coding sequence; or animal cell systems.

The expression elements of these systems vary in their strength and specificities. Depending on the host/vector system utilized, any of a number of suitable transcription and translation elements, including constitutive and inducible promoters, may be used in the expression vector. For example, when
5 cloning in bacterial systems, inducible promoters such as pL of bacteriophage λ , plac, ptrp, ptac (ptrp-lac hybrid promoter) and the like may be used; when
10 cloning in insect cell systems, promoters such as the baculovirus polyhedrin promoter may be used; when
cloning in plant cell systems, promoters derived from the genome of plant cells (e.g., heat shock promoters; the promoter for the small subunit of RUBISCO; the promoter for the chlorophyll a/b binding protein) or
15 from plant viruses (e.g., the 35S RNA promoter of CaMV; the coat protein promoter of TMV) may be used; when cloning in mammalian cell systems, promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses
20 (e.g., the adenovirus late promoter; the vaccinia virus 7.5K promoter) may be used; when generating cell lines that contain multiple copies of the tie-2 DNA, SV40-, BPV- and EBV-based vectors may be used with an appropriate selectable marker.

25 In bacterial systems a number of expression vectors may be advantageously selected depending upon the use intended for the tie-2 expressed. For example, when large quantities of tie-2 are to be produced for the generation of antibodies or to screen
30 peptide libraries, vectors which direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include but are not limited to the E. coli expression vector pUR278 (Ruther et al., 1983, EMBO J. 2:1791),
35 in which the tie-2 coding sequence may be ligated into the vector in frame with the lacZ coding region so

that a hybrid *tie-2/lacZ* protein is produced; pIN
vectors (Inouye & Inouye, 1985, Nucleic acids Res.
13:3101-3109; Van Heeke & Schuster, 1989, J. Biol.
Chem. 264:5503-5509); and the like. pGEX vectors may
also be used to express foreign polypeptides as fusion
proteins with glutathione S-transferase (GST). In
5 general, such fusion proteins are soluble and can
easily be purified from lysed cells by adsorption to
glutathione-agarose beads followed by elution in the
presence of free glutathione. The pGEX vectors are
designed to include thrombin or factor Xa protease
10 cleavage sites so that the cloned polypeptide of
interest can be released from the GST moiety.

In yeast, a number of vectors containing
constitutive or inducible promoters may be used. For
a review see, Current Protocols in Molecular Biology,
15 Vol. 2, 1988, Ed. Ausubel et al., Greene Publish.
Assoc. & Wiley Interscience, Ch. 13; Grant et al.,
1987, Expression and Secretion Vectors for Yeast, in
Methods in Enzymology, Eds. Wu & Grossman, 1987, Acad.
Press, N.Y., Vol. 153, pp. 516-544; Glover, 1986, DNA
20 Cloning, Vol. II, IRL Press, Wash., D.C., Ch. 3; and
Bitter, 1987, Heterologous Gene Expression in Yeast,
Methods in Enzymology, Eds. Berger & Kimmel, Acad.
Press, N.Y., Vol. 152, pp. 673-684; and The Molecular
Biology of the Yeast *Saccharomyces*, 1982, Eds.
25 Strathern et al., Cold Spring Harbor Press, Vols. I
and II.

In cases where plant expression vectors are used,
the expression of the *tie-2* coding sequence may be
driven by any of a number of promoters. For example,
30 viral promoters such as the 35S RNA and 19S RNA
promoters of CaMV (Brisson et al., 1984, Nature
310:511-514), or the coat protein promoter of TMV
(Takamatsu et al., 1987, EMBO J. 6:307-311) may be
used; alternatively, plant promoters such as the small
35 subunit of RUBISCO (Coruzzi et al., 1984, EMBO J.

3:1671-1680; Broglie et al., 1984, Science 224:838-843); or heat shock promoters, e.g., soybean hsp17.5-E or hsp17.3-B (Gurley et al., 1986, Mol. Cell. Biol. 6:559-565) may be used. These constructs can be introduced into plant cells using Ti plasmids, Ri plasmids, plant virus vectors, direct DNA transformation, microinjection, electroporation, etc. For reviews of such techniques see, for example, Weissbach & Weissbach, 1988, Methods for Plant Molecular Biology, Academic Press, NY, Section VIII, pp. 421-463; and Grierson & Corey, 1988, Plant Molecular Biology, 2d Ed., Blackie, London, Ch. 7-9.

An alternative expression system which could be used to express *tie-2* is an insect system. In one such system, Autographa californica nuclear polyhydrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in Spodoptera frugiperda cells. The *tie-2* coding sequence may be cloned into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter). Successful insertion of the *tie-2* coding sequence will result in inactivation of the polyhedrin gene and production of non-occluded recombinant virus (i.e., virus lacking the proteinaceous coat coded for by the polyhedrin gene). These recombinant viruses are then used to infect Spodoptera frugiperda cells in which the inserted gene is expressed. (E.g., see Smith et al., 1983, J. Virol. 46:584; Smith, U.S. Patent No. 4,215,051).

In mammalian host cells, a number of viral based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the *tie-2* coding sequence may be ligated to an adenovirus transcription/translation control complex, e.g., the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus

genome by in vitro or in vivo recombination. Insertion in a non-essential region of the viral genome (e.g., region E1 or E3) will result in a recombinant virus that is viable and capable of expressing *tie-2* in infected hosts. (E.g., See Logan & Shenk, 1984, Proc. Natl. Acad. Sci. (USA) 81:3655-3659). Alternatively, the vaccinia 7.5K promoter may be used. (See, e.g., Mackett et al., 1982, Proc. Natl. Acad. Sci. (USA) 79:7415-7419; Mackett et al., 1984, J. Virol. 49:857-864; Panicali et al., 1982, Proc. Natl. Acad. Sci. 79:4927-4931).

Specific initiation signals may also be required for efficient translation of inserted *tie-2* coding sequences. These signals include the ATG initiation codon and adjacent sequences. In cases where the entire *tie-2* gene, including its own initiation codon and adjacent sequences, is inserted into the appropriate expression vector, no additional translational control signals may be needed. However, in cases where only a portion of the *tie-2* coding sequence is inserted, exogenous translational control signals, including the ATG initiation codon, must be provided. Furthermore, the initiation codon must be in phase with the reading frame of the *tie-2* coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (see Bittner et al., 1987, Methods in Enzymol. 153:516-544).

In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (e.g., glycosylation) and processing (e.g., cleavage)

of protein products may be important for the function of the protein. The presence of nine consensus N-glycosylation sites in the *tie-2* extracellular domain indicate that proper modification may be important for *tie-2* function. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins.

Appropriate cells lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. To this end, eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include but are not limited to CHO, VERO, BHK, HeLa, COS, MDCK, 293, WI38, etc.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express the *tie-2* may be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with the *tie-2* DNA controlled by appropriate expression control elements (e.g., promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. This method may advantageously be used to engineer cell lines which express the *tie-2* on the cell surface. Such engineered cell lines are

particularly useful in screening for drugs that affect tie-2.

A number of selection systems may be used, including but not limited to the herpes simplex virus thymidine kinase (Wigler, et al., 1977, Cell 11:223), hypoxanthine-guanine phosphoribosyltransferase
5 (Szybalska & Szybalski, 1962, Proc. Natl. Acad. Sci. USA 48:2026), and adenine phosphoribosyltransferase (Lowy, et al., 1980, Cell 22:817) genes can be employed in tk⁻, hgp^rt⁻ or ap^rt⁻ cells, respectively. Also, antimetabolite resistance can be used as the
10 basis of selection for dhfr, which confers resistance to methotrexate (Wigler, et al., 1980, Natl. Acad. Sci. USA 77:3567; O'Hare, et al., 1981, Proc. Natl. Acad. Sci. USA 78:1527); gpt, which confers resistance to mycophenolic acid (Mulligan & Berg, 1981), Proc.
15 Natl. Acad. Sci. USA 78:2072); neo, which confers resistance to the aminoglycoside G-418 (Colberre-Garapin, et al., 1981, J. Mol. Biol. 150:1); and hyg^r, which confers resistance to hygromycin (Santerre, et al., 1984, Gene 30:147) genes.
20 Recently, additional selectable genes have been described, namely trpB, which allows cells to utilize indole in place of tryptophan; hisD, which allows cells to utilize histinol in place of histidine (Hartman & Mulligan, 1988, Proc. Natl. Acad. Sci. USA
25 85:8047); and ODC (ornithine decarboxylase) which confers resistance to the ornithine decarboxylase inhibitor, 2-(difluoromethyl)-DL-ornithine, DFMO (McConlogue L., 1987, In: Current Communications in Molecular Biology, Cold Spring Harbor Laboratory ed.).
30

5.2.2. IDENTIFICATION OF TRANSFECTANTS OR TRANSFORMANTS THAT EXPRESS THE TIE-2

The host cells which contain the coding sequence
35 and which express the biologically active gene product

may be identified by at least four general approaches;
(a) DNA-DNA or DNA-RNA hybridization; (b) the presence
or absence of "marker" gene functions; (c) assessing
the level of transcription as measured by the expres-
sion of *tie-2* mRNA transcripts in the host cell; and
5 (d) detection of the gene product as measured by
immunoassay or by its biological activity.

In the first approach, the presence of the *tie-2*
coding sequence inserted in the expression vector can
be detected by DNA-DNA or DNA-RNA hybridization using
10 probes comprising nucleotide sequences that are
homologous to the *tie-2* coding sequence, respectively,
or portions or derivatives thereof.

In the second approach, the recombinant expres-
sion vector/host system can be identified and selected
based upon the presence or absence of certain "marker"
15 gene functions (e.g., thymidine kinase activity,
resistance to antibiotics, resistance to methotrexate,
transformation phenotype, occlusion body formation in
baculovirus, etc.). For example, if the *tie-2* coding
sequence is inserted within a marker gene sequence of
20 the vector, recombinants containing the *tie-2* coding
sequence can be identified by the absence of the
marker gene function. Alternatively, a marker gene
can be placed in tandem with the *tie-2* sequence under
the control of the same or different promoter used to
25 control the expression of the *tie-2* coding sequence.
Expression of the marker in response to induction or
selection indicates expression of the *tie-2* coding
sequence.

In the third approach, transcriptional activity
30 for the *tie-2* coding region can be assessed by
hybridization assays. For example, RNA can be
isolated and analyzed by Northern blot using a probe
homologous to the *tie-2* coding sequence or particular
portions thereof. Alternatively, total nucleic acids
35

of the host cell may be extracted and assayed for hybridization to such probes.

In the fourth approach, the expression of the *tie-2* protein product can be assessed immunologically, for example by Western blots, immunoassays such as radioimmuno-precipitation, enzyme-linked immunoassays and the like.

5.3. USES OF THE *TIE-2* RECEPTOR AND ENGINEERED CELL LINES

The uses of the *tie-2* receptor and engineered cell lines, described in the subsections below, may be employed equally well for the *tie-2* family of receptor tyrosine kinases.

In an embodiment of the invention, engineered cell lines which express the entire *tie-2* coding region or its ligand binding domain may be utilized to screen and identify the natural ligand and/or ligand antagonists as well as agonists. Synthetic compounds, natural products, and other sources of potentially biologically active materials can be screened in a number of ways.

5.3.1 IDENTIFICATION OF *TIE-2* LIGAND USING ENGINEERED CELL LINES

Engineered cell lines that express the *tie-2* receptor, may be advantageously used to identify and characterize the natural *tie-2* ligand(s). For example, a genomic or cDNA library may be transfected into the engineered cell lines. Any cell that expresses and secretes the *tie-2* ligand, due to the transfer of a DNA clone capable of encoding the *tie-2* ligand, will stimulate the biological activity of the recombinantly expressed the *tie-2* receptor. The resulting autocrine loop will lead to preferential proliferation of cells expressing *tie-2* ligand and the

resulting cell colonies may be used to isolate the DNA encoding the *tie-2* ligand.

The engineered cell lines may also be used to assay tissue or cell extracts for their ability to activate *tie-2* receptor tyrosine kinase activity. Activation of *tie-2* tyrosine kinase activity may be
5 assayed using a variety of methods. For example, in cells overexpressing the *tie-2* receptor, overall incorporation of labeled PO_4 into the cell may be measured after contacting cells with extracts.

Alternatively, the *tie-2* protein may be
10 immunoprecipitated following stimulation with extracts, using anti-*tie-2* antibodies, followed by Western blot analysis using anti-tyrosine phosphate antibodies to determine whether the immunoprecipitated
15 *tie-2* has been tyrosine phosphorylated.

Once an extract is identified which contains *tie-2* ligand, various procedures and techniques known in the art which include but are not limited to chromatography (e.g., reverse phase liquid, gell
20 permeation, liquid exchange, ion exchange, size exclusion, affinity chromatography), centrifugation, electrophoretic procedures, differential solubility, or other standard techniques may be used to purify
25 *tie-2* ligand.

In a preferred embodiment, affinity
25 chromatography using recombinantly expressed *tie-2* covalently attached to a column matrix may be used to purify *tie-2* ligand. Alternatively, a recombinantly expressed hybrid protein comprised of the *tie-2*
30 extracellular domain fused to the immunoglobulin protein A binding domain may be used to purify *tie-2* ligand. The fusion protein may be used to prepare a column matrix over which cell extracts may be added, or, the fusion protein may be used to
35 immunoprecipitate the *tie-2* ligand from cell extracts.

Once purified, the *tie-2* ligand may be subjected to microsequencing, using techniques routinely used by those skilled in the art to determine the amino acid sequence of a protein. If the *tie-2* ligand molecule is blocked at the amino terminus, the protein may be chemically cleaved or partially enzymatically digested to yield peptide fragments that may be purified and sequenced.

A mixture of degenerate oligonucleotide probes may be designed using the information derived from the protein sequencing of the purified *tie-2* ligand. The oligonucleotides may be labeled and used directly to screen a cDNA library for clones containing inserts with sequence homology to the oligonucleotide sequences. Alternatively, the oligonucleotides may be used as primers in a polymerase chain reaction. The amplified DNA fragment may be labeled and used to screen a library for isolation of full length clones.

5.3.2. SCREENING OF PEPTIDE LIBRARY WITH *TIE-2* PROTEIN OR ENGINEERED CELL LINES

Random peptide libraries consisting of all possible combinations of amino acids attached to a solid phase support may be used to identify peptides that are able to bind to the ligand binding site of a given receptor or other functional domains of a receptor such as kinase domains (Lam, K.S. et al., 1991, Nature 354: 82-84). The screening of peptide libraries may have therapeutic value in the discovery of pharmaceutical agents that act to inhibit the biological activity of receptors through their interactions with the given receptor.

Identification of molecules that are able to bind to the *tie-2* may be accomplished by screening a peptide library with recombinant soluble *tie-2* protein. Methods for expression and purification of

tie-2 are described in Section 5.2.1 and may be used to express recombinant full length *tie-2* or fragments of *tie-2* depending on the functional domains of interest. For example, the kinase and extracellular ligand binding domains of *tie-2* may be separately expressed and used to screen peptide libraries.

To identify and isolate the peptide/solid phase support that interacts and forms a complex with *tie-2*, it is necessary to label or "tag" the *tie-2* molecule. The *tie-2* protein may be conjugated to enzymes such as alkaline phosphatase or horseradish peroxidase or to other reagents such as fluorescent labels which may include fluorescein isothiocyanate (FITC), phycoerythrin (PE) or rhodamine. Conjugation of any given label, to *tie-2*, may be performed using techniques that are routine in the art.

Alternatively, *tie-2* expression vectors may be engineered to express a chimeric *tie-2* protein containing an epitope for which a commercially available antibody exist. The epitope specific antibody may be tagged using methods well known in the art including labeling with enzymes, fluorescent dyes or colored or magnetic beads.

To screen a peptide library the "tagged" *tie-2* conjugate is incubated with the random peptide library for 30 minutes to one hour at 22°C to allow complex formation between *tie-2* and peptide species within the library. The library is then washed to remove any unbound *tie-2* protein. If *tie-2* has been conjugated to alkaline phosphatase or horseradish peroxidase the whole library is poured into a petri dish containing substrates for either alkaline phosphatase or peroxidase, for example, 5-bromo-4-chloro-3-indoyl phosphate (BCIP) or 3,3',4,4"-diaminobenzidine (DAB), respectively. After incubating for several minutes, the peptide/solid phase-*tie-2* complex changes color, and can be easily identified and isolated physically

under a dissecting microscope with a micromanipulator. If a fluorescent tagged *tie-2* molecule has been used, complexes may be isolated by fluorescent activated sorting. If a chimeric *tie-2* protein expressing a heterologous epitope has been used, detection of the peptide/*tie-2* complex may be accomplished by using a
5 labeled epitope specific antibody. Once isolated, the identity of the peptide attached to the solid phase support may be determined by peptide sequencing.

In addition to using soluble *tie-2* molecules, in
10 another embodiment, it is possible to detect peptides that bind to cell surface receptors using intact cells. The use of intact cells is preferred for use with receptors that are multi-subunits or labile or with receptors that require the lipid domain of the
15 cell membrane to be functional. Methods for generating cell lines expressing *tie-2* are described in Sections 5.2.1. and 5.2.2. The cells used in this technique may be either live or fixed cells. The cells will be incubated with the random peptide
20 library and will bind to certain peptides in the library to form a "rosette" between the target cells and the relevant solid phase support/peptide. The rosette can thereafter be isolated by differential centrifugation or removed physically under a
25 dissecting microscope.

As an alternative to whole cell assays for
membrane bound receptors or receptors that require the lipid domain of the cell membrane to be functional, the receptor molecules can be reconstituted into
30 liposomes where label or "tag" can be attached.

5.3.3: ANTIBODY PRODUCTION AND SCREENING

Various procedures known in the art may be used for the production of antibodies to epitopes of the
35 recombinantly produced *tie-2* receptor. Such

antibodies include but are not limited to polyclonal, monoclonal, chimeric, single chain, Fab fragments and fragments produced by an Fab expression library. Neutralizing antibodies i.e., those which compete for the ligand binding site of the receptor are especially preferred for diagnostics and therapeutics.

5 Monoclonal antibodies that bind *tie-2* may be radioactively labeled allowing one to follow their location and distribution in the body after injection. Radioactivity tagged antibodies may be used as a non-invasive diagnostic tool for imaging *de novo* cells
10 expressing *tie-2*.

 Immunotoxins may also be designed which target cytotoxic agents to specific sites in the body. For example, high affinity *tie-2* specific monoclonal
15 antibodies may be covalently complexed to bacterial or plant toxins, such as diphtheria toxin, abrin or ricin. A general method of preparation of antibody/hybrid molecules may involve use of thiol-crosslinking reagents such as SPDP, which attack the
20 primary amino groups on the antibody and by disulfide exchange, attach the toxin to the antibody. The hybrid antibodies may be used to specifically eliminate *tie-2* expressing cells.

 For the production of antibodies, various host
25 animals may be immunized by injection with the *tie-2* protein including but not limited to rabbits, mice, rats, etc. Various adjuvants may be used to increase the immunological response, depending on the host species, including but not limited to Freund's
30 (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, dinitrophenol, and potentially useful human adjuvants
35 such as BCG (bacilli Calmette-Guerin) and Corynebacterium parvum.

Monoclonal antibodies to tie-2 may be prepared by using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include but are not limited to the hybridoma technique originally described by Kohler and Milstein, (Nature, 1975, 256:495-497), the human B-cell hybridoma technique (Kosbor et al., 1983, Immunology Today, 4:72; Cote et al., 1983, Proc. Natl. Acad. Sci., 80:2026-2030) and the EBV-hybridoma technique (Cole et al., 1985, Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96). In addition, techniques developed for the production of "chimeric antibodies" (Morrison et al., 1984, Proc. Natl. Acad. Sci., 81:6851-6855; Neuberger et al., 1984, Nature, 312:604-608; Takeda et al., 1985, Nature, 314:452-454) by splicing the genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used. Alternatively, techniques described for the production of single chain antibodies (U.S. Patent 4,946,778) can be adapted to produce tie-2-specific single chain antibodies.

Antibody fragments which contain specific binding sites of tie-2 may be generated by known techniques. For example, such fragments include but are not limited to: the F(ab')₂ fragments which can be produced by pepsin digestion of the antibody molecule and the Fab fragments which can be generated by reducing the disulfide bridges of the F(ab')₂ fragments. Alternatively, Fab expression libraries may be constructed (Huse et al., 1989, Science, 246:1275-1281) to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity to tie-2.

35

5.4. USES OF TIE-2 CODING SEQUENCE

The tie-2 coding sequence may be used for diagnostic purposes for detection of tie-2 expression. Included in the scope of the invention are oligoribonucleotide sequences, that include antisense RNA and DNA molecules and ribozymes that function to
5 inhibit translation of tie-2. In addition, mutated forms of tie-2, having a dominant negative effect, may be expressed in targeted cell populations to inhibit the activity of endogenously expressed tie-2.

5.4.1. USE OF TIE-2 CODING SEQUENCE IN DIAGNOSTICS AND THERAPEUTICS

The tie-2 DNA may have a number of uses for the diagnosis of diseases resulting from aberrant
15 expression of tie-2. For example, the tie-2 DNA sequence may be used in hybridization assays of biopsies or autopsies to diagnose abnormalities of tie-2 expression; e.g., Southern or Northern analysis, including *in situ* hybridization assays.

Also within the scope of the invention are oligo-
20 ribonucleotide sequences, that include anti-sense RNA and DNA molecules and ribozymes that function to inhibit the translation of tie-2 mRNA. Anti-sense RNA and DNA molecules act to directly block the translation of mRNA by binding to targeted mRNA and
25 preventing protein translation. In regard to antisense DNA, oligodeoxyribonucleotides derived from the translation initiation site, e.g., between -10 and +10 regions of the tie-2 nucleotide sequence, are preferred.

30 Ribozymes are enzymatic RNA molecules capable of catalyzing the specific cleavage of RNA. The mechanism of ribozyme action involves sequence specific hybridization of the ribozyme molecule to complementary target RNA, followed by an endonucleolytic cleavage.
35 Within the scope of the invention are engineered

hammerhead motif ribozyme molecules that specifically and efficiently catalyze endonucleolytic cleavage of tie-2 RNA sequences.

Specific ribozyme cleavage sites within any potential RNA target are initially identified by scanning the target molecule for ribozyme cleavage sites which include the following sequences, GUA, GUU and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides corresponding to the region of the target gene containing the cleavage site may be evaluated for predicted structural features such as secondary structure that may render the oligonucleotide sequence unsuitable. The suitability of candidate targets may also be evaluated by testing their accessibility to hybridization with complementary oligonucleotides, using ribonuclease protection assays.

Both anti-sense RNA and DNA molecules and ribozymes of the invention may be prepared by any method known in the art for the synthesis of RNA molecules. These include techniques for chemically synthesizing oligodeoxyribonucleotides well known in the art such as for example solid phase phosphoramidite chemical synthesis. Alternatively, RNA molecules may be generated by *in vitro* and *in vivo* transcription of DNA sequences encoding the antisense RNA molecule. Such DNA sequences may be incorporated into a wide variety of vectors which incorporate suitable RNA polymerase promoters such as the T7 or SP6 polymerase promoters. Alternatively, antisense cDNA constructs that synthesize antisense RNA constitutively or inducibly, depending on the promoter used, can be introduced stably into cell lines.

Various modifications to the DNA molecules may be introduced as a means of increasing intracellular stability and half-life. Possible modifications include but are not limited to the addition of

flanking sequences of ribo- or deoxy- nucleotides to the 5' and/or 3' ends of the molecule or the use of phosphorothioate or 2' O-methyl rather than phosphodiesterase linkages within the oligodeoxyribonucleotide backbone.

5

5.4.2. USE OF DOMINANT NEGATIVE
TIE-2 MUTANTS IN GENE THERAPY

Receptor dimerization induced by ligands, is thought to provide an allosteric regulatory signal that functions to couple ligand binding to stimulation of kinase activity. Defective receptors can function as dominant negative mutations by suppressing the activation and response of normal receptors by formation of unproductive heterodimers. Therefore, defective receptors can be engineered into recombinant viral vectors and used in gene therapy in individuals that inappropriately express tie-2.

In an embodiment of the invention, mutant forms of the tie-2 molecule having a dominant negative effect may be identified by expression in selected cells. Deletion or missense mutants of tie-2 that retain the ability to form dimers with wild type tie-2 protein but cannot function in signal transduction may be used to inhibit the biological activity of the endogenous wild type tie-2. For example, the cytoplasmic kinase domain of tie-2 may be deleted resulting in a truncated tie-2 molecule that is still able to undergo dimerization with endogenous wild type receptors but unable to transduce a signal.

Recombinant viruses may be engineered to express dominant negative forms of tie-2 which may be used to inhibit the activity of the wild type endogenous tie-2. These viruses may be used therapeutically for treatment of diseases resulting from aberrant expression or activity of tie-2, such as cancers.

Expression vectors derived from viruses such as retroviruses, vaccinia virus, adeno-associated virus, herpes viruses, or bovine papilloma virus, may be used for delivery of recombinant *tie-2* into the targeted cell population. Methods which are well known to those skilled in the art can be used to construct those recombinant viral vectors containing *tie-2* coding sequence. See, for example, the techniques described in Maniatis et al., 1989, Molecular Cloning A Laboratory Manual, Cold Spring Harbor Laboratory, N.Y. and Ausubel et al., 1989, Current Protocols in Molecular Biology, Greene Publishing Associates and Wiley Interscience, N.Y. Alternatively, recombinant *tie-2* molecules can be reconstituted into liposomes for delivery to target cells.

6. EXAMPLES: CLONING AND CHARACTER-
IZATION OF THE *TIE-2*
RECEPTOR TYROSINE KINASE

6.1. MATERIALS AND METHODS

6.1.1. ANIMALS AND TISSUES

Balb/c mice were mated overnight and the morning of vaginal plug detection was defined as 0.5 day of gestation. Organs were removed, frozen immediately in liquid nitrogen and stored at -80°C. Capillary fragments from pooled P4-P8 mice brains were prepared according to Risau et al. (1990 J. Cell Biol. 110:1757-1766). For *in situ* hybridization and immunohistochemistry, whole embryos or organs were fixed for 12 hours in freshly prepared 4% paraformaldehyde in PBS at 4°C, rinsed for 24-48 hours in 0.5 M sucrose at PBS at 4°C, embedded in TissueTek (Miles) and stored frozen at -80°C.

6.1.2. RNA EXTRACTION AND ANALYSIS

Total cytoplasmic RNA was isolated according to the method of Chomczynski and Sacchi (1987 Anal. Biochem. 162:156-159). Enrichment for poly(A)⁺ containing fractions was achieved by oligo(dT) chromatography using push columns (Stratagene).
 5 Aliquots of poly(A)⁺ RNA were electrophoresed in agarose gels containing 0.66 M formaldehyde and transferred to Zeta-Probe membrane (Bio Rad) in 20x SSPE. Hybridizations were performed overnight in 0.5
 10 M sodium phosphate buffer, 5% SDS, 1% BSA, pH 7.5 at 68°C with 1x10⁶ cts/minute/ml of probe, which had been labelled with ³²P-dCTP according to the protocol of a random-primed DNA-labeling kit (Boehringer Mannheim). Membranes were washed under high-stringency conditions
 15 at 68°C in 0.1XSSPE. 0.5% SDS and autoradiographed at -80°C on Fuji films. Poly(A)⁺ RNA from brain capillary fragments was isolated using a QuickPrep Micro mRNA purification kit from Pharmacia.

6.1.3. PCR AND cDNA CLONING

20 Capillary poly(A)⁺ RNA was reverse transcribed and aliquots of the cDNA were used as templates in PCR reactions. The following oligonucleotides were used as primers: 5'-CAC/TCGIGAC/TC/TTIGCIGCIA/CG-3', 5'-
 25 AC/TICCIAA/CIC/GA/TCCAIACA/CTC-3' (I stands for inosine) in addition to the following premiss described by Wilks et al. (1989, DPNAS Proc. Natl. Acad. Sci. USA 86:1603-1607):

5'-CGGATCCACAGNGACCT-3' and
 C TT

30 3'-CTGCAGACCAGGAAACCTTAAGG-5'
 A C T

The amplification products were separated in acrylamide gels. Fragments of the expected size were purified, labeled and used as probes to screen a
 35 random hexanucleotide-primed cDNA library constructed

with a Time Saver cDNA Synthesis Kit (Pharmacia).
cDNA fragments were subcloned into Bluescript vectors
(Stratagene). Sequencing was done by using nested
oligonucleotide primers in combination with a 373 DNA
Sequencer (Applied Biosystems) and with the Convention
Sequenase System (USB).

5

6.1.4. IN SITU HYBRIDIZATION

Preparation of tissue sections and *in situ*
hybridizations with single-stranded DNA probes or
single-stranded RNA probes was performed as described
by Schnürch and Risau (1991, Development 111:1143-
1154). The probe for all hybridization experiments
was derived from a 1179 bp DNA fragment encoding a
portion of the putative tie-2 protein from amino acid
419 to amino acid 812.

10

15

6.1.5. IMMUNOHISTOCHEMISTRY

Embedded embryos and whole organs were sectioned
on a Leitz cryostat 8 μ m sections were mounted on
organosilane-treated slides, dried overnight under
vacuum and stored desiccated at -80°C. Sections were
brought to room temperature, rehydrated in PBS for 5
minutes and incubated in 0.1% H₂O₂ in methanol for 15
minutes. After washing three times in PBS for 5
minutes each, nonspecific antibody binding was blocked
by application of 20% normal goat serum in PBS for 20
minutes. Sections were washed, incubated with a rat
monoclonal antibody against mouse PECAM for one hour,
washed again and then incubated with a viotinylated
gout anti-rat IgG (Dianova) for one hours. Color
development was performed with a Vectastain ABC Kit
(Vector Laboratories) according to the vendor's
protocol. Sections were slightly counterstained with
toluene blue, dehydrated and mounted.

20

25

30

35

6.2. RESULTS

6.2.1. cDNA CLONING AND STRUCTURE OF THE *TIE-2* PROTEIN

The approach for the isolation of receptor tyrosine kinases expressed in endothelial cells of sprouting blood vessels involved the following steps.

5 First, mRNA was purified from capillaries of pooled P4-P8 mice brains. A portion of the mRNA was reverse transcribed and used in PCR reactions with degenerate primers deduced from conserved protein regions of tyrosine kinases. Gel-purified reaction products of
10 the expected size were radiolabelled and used directly as hybridization probes to screen a cDNA library constructed from the remainder of the capillary mRNA.

Partial sequencing of the inserts from positive phages revealed that 13 of the cDNAs were derived from
15 one mRNA species. The longest of these 13 cDNAs, a fragment of 4640 bp, was sequenced completely (FIG. 1A, 1B, 1C). It contained a long open reading frame encoding a protein of 1123 amino acid residues (FIG. 2A). This deduced polypeptide has all features of a
20 receptor tyrosine kinase: an amino terminal signal sequence followed by a long extracellular domain, a single hydrophobic transmembrane region and a cytoplasmic portion that contains a tyrosine kinase domain (Fig. 2B).

25 A survey for homologous proteins revealed that the predicted protein is most closely related to the two recently identified tyrosine kinases *tie* and *tek* (Partanen et al., 1992 Mol. Cell. Biol. 12:1698-1707; Dumont et al., 1992 Oncogene 7:1471-1480). With the
30 exception of two residues, the protein sequence published for *tek* is identical to the intracellular part of our polypeptide, from position 823 to the carboxyl-terminal residue 1123. Both an extracellular domain and a transmembrane region are missing in the
35 *tek* polypeptide (Fig. 3). It is therefore possible

that *tek* represents a partial sequence or that the mRNA encoding *tek* is the result of an alternative splice event, which results in the production of a cytoplasmic tyrosine kinase. Comparison of our protein with *tie* reveals a high degree of similarity especially in the cytoplasmic part (76% sequence identify). In the extracellular domain and the transmembrane region, the similarity is less pronounced (33% and 37% respectively; Fig. 3). Ziegler et al. (1993 *Oncogene* 8:663-670) have also reported the cloning of a cDNA whose translation product is a receptor tyrosine kinase with an overall similarity of more than 90% when compared to *tie-2* (Fig. 3). It is therefore likely that this protein, which they called *TEK*, is the human homolog of *tie-2*. The intracellular part of *tie-2* is also related to the product of the human *ret* protooncogene and to FGF receptors (Takahashi et al., 1989 *Oncogene* 4:805-806; Partanen et al., 1991 *EMBO J.* 10:1347-1354; Stark et al., 1991 *Development* 113:641-651). The similarity to both is about 43%. The intracellular part can be divided into three characteristic regions: the juxtamembrane sequence, the catalytic domain and the cytoplasmic tail. The kinase domain, which is split by an 14 amino acid insertion contains the GxGxxG consensus sequence that is part of the ATP binding site. Typical tyrosine kinase motifs like HRDLAARN and DFGL are present.

Within the extracellular part, the FASTA program detected homologies to proteins including TAN-1 (Ellisen et al., 1991 *Cell* 66:649-661), Xotch (Coffman et al., 1990 *Science* 249:1438-1440), Laminin (Sasaki et al., 1988 *J. Biol. Chem.* 263:16536-16544). Delta (Vässin et al., 1987 *EMBO J.* 6:3431-3440) and Perlecan (Noonan et al., 1991 *J. Biol. Chem.* 266:22939-22947) (around 30% sequence identity). The structural basis for these homologies are three EGF-like repeats in the

center of the extracellular portion of *tie-2* (Fig. 2A and 2B). EGF-like repeats consist of 30 to 40 amino acid residues often found in the extracellular parts of membrane-bound proteins or secreted proteins (Davies, 1990 New Biol. 5:410-419). A common feature of these domains are six conserved cysteine residues, which are known to be involved in disulfide bonds. The three EGF domains in *tie-2* contain two additional cysteine residues at the carboxyl-terminal end of each repeat.

The central EGF-like repeats are flanked by two different structural motifs, a single amino-terminal immunoglobulin (Ig) domain and a carboxyl-terminal triplet of fibronectin (FN) type III domains. Although the Ig domain does not exhibit clear sequence similarities to other known proteins (except for *tie* and *TEK*), the Ig domain has the conserved features of a C2-set domain including typical residues surrounding the first cysteine residue and the canonical GxYxC found at the second cysteine residue (Williams and Barclay, 1988 Ann. Rev. Immunol. 6:381-405).

The three fibronectin type III (FN III) domains are most closely related to FN III repeats present in the protein-tyrosine phosphatases Delta (Krueger et al., 1990 EMBO J. 9:3241-3252), LAR (Streuli et al., 1988 J. Exp. Med. 168:1553-1562) and DLAR (Streuli et al., 1989) and to FN III repeats of the axonal glycoprotein TAG-1 (Furley et al., 1990 Cell 61:157-170) (around 20% sequence identity). These domains have been described as units of approximately 90 amino acids containing hydrophobic residues at characteristic positions. In addition, a total of nine potential N-glycosylation sites are located in the extracellular part of *tie-2*.

6.2.2. TIE-2 EXPRESSION IN BRAIN CAPILLARIES

The amount of *tie-2* in mRNA from P4-P8 brain capillaries was compared with total P4 brain by Northern analysis. To avoid artifactual results, the part of the cDNA that encodes the least conserved regions of the protein, namely the three FN III domains, the transmembrane region and the juxtamembrane portion was used. Fig. 4 demonstrates the presence of a 4.7 kd mRNA highly enriched in the capillary fraction. RNA expression was also detected in organs like brain, kidney and heart.

6.2.3. TIE-2 EXPRESSION DURING BRAIN DEVELOPMENT

Investigation of the spatial and temporal expression profile of *tie-2* during brain development was performed by in situ hybridization. When analyzed, it became evident that *tie-2* mRNA is exclusively synthesized in the vasculature of (Fig. 5A-H) adult brain, P4 brain, E12.5 brain and E8.5 neuroectoderm. No other brain components were labelled. In P4 brain, *tie-2* expression was detected in capillaries that are about to invade the neural tissue as well as in vessels that have already reached deeper layers of the neuroectoderm. High magnifications in Fig. 5G,H clearly show the high concentration of silver grains over the vascular perikarya. In addition to capillaries, meningeal blood vessels and the choroid plexi were also found to synthesize *tie-2* mRNA at comparable levels (Figs 5, 7B). At 312.5 the overall expression pattern was virtually identical to that observed at P4, although the density of labelled structures in E12.5 brain was reduced (Fig. 5C,D). This observation correlates with the less extensive vascularization of the embryonic brain (Bär, 1980 Adv. Anat. Embryol. Cell Biol 59:1-

62). No hybridization signals were observed in the neuroectoderm of E8.5 embryos, because at that stage this tissue is still avascular (Fig. 8). In the adult brain, *tie-2* expression was detectable, although it seemed to be reduced when compared to postnatal or embryonic stages. Hybridization signals persisted over larger vessels especially those of the meninges (Fig. 5A,B). The synthesis of *tie-2* mRNA in smaller vessels and capillaries was barely detectable.

6.2.4. ENDOTHELIAL CELL-SPECIFIC EXPRESSION OF *TIE-2*

To identify the cellular source of *tie-2* mRNA, *in situ* hybridization and immunohistochemistry on adjacent sections were performed. The pattern of *tie-2* hybridization signals was compared with the immunohistochemical staining of a monoclonal antibody against the endothelial cell-specific adhesion molecule PECAM (CD31) (Newman et al., 1990, Science 247:1219-1222). Fig. 6 shows a representative example of one such experiment. The antibody stains the continuous layer of endothelial cells surrounding the lumen of a medium-sized blood vessel in the head region of an E12.5 embryo. On the adjacent section, the *tie-2*-specific probe labels the vessel in an identical way. These results, together with the northern hybridization signal detected in RNA from a capillary fraction highly enriched for endothelial cells, provide strong evidence for endothelium-specific expression of *tie-2* and PECAM in several organs and tissues. It is clear that *tie-2* mRNA is present in endothelial cells all over the body. Strong hybridization signals were associated with the heart endocardium as well with the myocardial blood vessels (Fig. 7E,F). The same holds true for the endothelium of the dorsal aorta (Fig. 7C,D), the intersomitic vasculature, the vessels surrounding the

lung bronchia and the capillaries perforating the spinal cord (Fig. 7H,D). In summary, *tie-2* gene expression seems to be a general feature of endothelial cells.

5 6.2.5. *TIE-2* EXPRESSION DURING
 EARLY STAGES OF DEVELOPMENT

10 The possible role of *tie-2* in early stages of
 vascular development was investigated by *in situ*
 hybridization studies with E8.5 sections. The probe
 detected *tie-2* mRNA in the mesodermal component of the
15 yolk sac (Fig. 8). This finding is of particular
 interest, because it is in the mesodermal component of
 the yolk sac that the first signs of blood vessel
 development are evident. Clusters of mesenchymal
15 cells form the so-called blood islands. At the margin
 of these aggregates cells, the so-called angioblasts,
 adopt an endothelial-like phenotype, whereas in the
 center cells differentiate into embryonic hemoblasts.
 Fig. 8 demonstrates that the peripheral angioblasts
20 synthesize high levels of *tie-2* mRNA.

20 Within the embryo proper, expression was seen in
 the anlagen of the vascular system, e.g. in the
 developing endocardium, the dorsal aortae and the
 cardinal veins (Fig. 8A,B). It is likely that these
25 signals stem from intraembryonic endothelial cell
 precursors, which are present in these structures at
 that stage, *tie-2* expression could also be detected in
 the allantois and in blood vessels of the maternal
 decidua.

30

35

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Risau, Werner
Schnurch, Harald
- (ii) TITLE OF INVENTION: TIE-2 A NOVEL TYROSINE RECEPTOR KINASE
- (iii) NUMBER OF SEQUENCES: 5
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: Pennie & Edmonds
 - (B) STREET: 1155 Avenue of the Americas
 - (C) CITY: New York
 - (D) STATE: New York
 - (E) COUNTRY: USA
 - (F) ZIP: 10036-2711
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER: US 08/152,552
 - (B) FILING DATE: 12-NOV-1993
 - (C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Coruzzi, Laura A.
 - (B) REGISTRATION NUMBER: 30,742
 - (C) REFERENCE/DOCKET NUMBER: 7683-059
- (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: (212) 790-9090
 - (B) TELEFAX: (212) 869-9741
 - (C) TELEX: 66141 PENNIE

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 4640 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

- (ix) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) LOCATION: 341..3712

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

| | |
|---|-----|
| AGCAGGAGCC GGAGCAGGAG CAGAAGATAA GCCTTGGATG AAGGGCAAGA TGGATAGGGC | 60 |
| TCGCTCTGCC CCAAGCCCTG CTGATACCAA GTGCCTTTAA GATACAGCCT TTCCCATCCT | 120 |
| AATCTGCAAA GGAAACAGGA AAAAGGAACT TAACCCTCCC TGTGCTCAGA CAGAAATGAG | 180 |

| | | | | | | |
|------------|------------|-------------|------------|------------|------------|------|
| ACTGTTACCG | CCTGCTTCTG | TGGTGTCTTCT | CCTTGCCGCC | AACTTGTAAG | CAAGAGCGAG | 240 |
| TGGACCATGC | GAGCGGGAAG | TCGCAAAAGT | GTGAGTTGTT | GAAAGCTTCC | CAGGGACTCA | 300 |
| TGCTCATCTG | TGGACGCTGG | ATGGGGAGAT | CTGGGGAAGT | ATGGACTCTT | TAGCCGGCTT | 360 |
| AGTTCTCTGT | GGAGTCAGCT | TGCTCCTTTA | TGGAGTAGTA | GAAGGTGCCA | TGGACCTGAT | 420 |
| CTTGATCAAT | TCCCTACCTC | TTGTGTCTGA | TGCCGAAACA | TCCCTCACCT | GCATTGCCTC | 480 |
| TGGGTGGCAC | CCCCATGAGC | CCATCACCAT | AGGAAGGGAC | TTTGAAGCCT | TAATGAACCA | 540 |
| GCACCAAGAT | CCACTGGAGG | TTACTCAAGA | TGTGACCAGA | GAATGGGCGA | AAAAAGTTGT | 600 |
| TTGGAAGAGA | GAAAAGGCCA | GTAAGATTAA | TGGTGCTTAT | TTCTGTGAAG | GTCGAGTTCG | 660 |
| AGGACAGGCT | ATAAGGATAC | GGACCATGAA | GATGCGTCAA | CAAGCGTCCT | TCCTACCTGC | 720 |
| TACTTTAACT | ATGACCGTGG | ACAGGGGAGA | TAATGTGAAC | ATATCTTTCA | AAAAGGTGTT | 780 |
| AATTAAAGAA | GAAGATGCAG | TGATTTACAA | AAATGGCTCC | TTCATCCACT | CAGTGCCCCG | 840 |
| GCATGAAGTA | CCTGATATTT | TAGAAGTTCA | CTTGCCGCAT | GCTCAGCCCC | AGGATGCTGG | 900 |
| TGTGTACTCG | GCCAGGTACA | TAGGAGGAAA | CCTGTTTACC | TCAGCCTTCA | CCAGGCTGAT | 960 |
| TGTTCCGAGA | TGTGAAGCTC | AGAAGTGGGG | GCCCGACTGT | AGCCGTCCTT | GTACTACTTG | 1020 |
| CAAGAACAAT | GGAGTCTGCC | ATGAAGATAC | CGGGGAATGC | ATTTGCCCTC | CTGGGTTTAT | 1080 |
| GGGGAGAACA | TGTGAGAAAG | CTTGTGAGCC | GCACACATTT | GGCAGGACCT | GTAAAGAAAG | 1140 |
| GTGTAGTGGA | CCAGAAGGAT | GCAAGTCTTA | TGTGTTCTGT | CTCCCAGACC | CTTACGGGTG | 1200 |
| TTCCTGTGCC | ACAGGCTGGA | GGGGGTGCA | GTGCAATGAA | GCATGCCCAT | CTGGTTACTA | 1260 |
| CGGACCAGAC | TGTAAGCTCA | GGTGCCACTG | TACCAATGAA | GAGATATGTG | ATCGGTTCCA | 1320 |
| AGGATGCCTC | TGCTCTCAAG | GATGGCAAGG | GCTGCAGTGT | GAGAAAGAAG | GCAGGCCAAG | 1380 |
| GATGACTCCA | CAGATAGAGG | ATTTGCCAGA | TCACATTGAA | GTAAACAGTG | GAAAATTTAA | 1440 |
| CCCCATCTGC | AAAGCCTCTG | GGTGGCCACT | ACCTACTAGT | GAAGAAATGA | CCCTAGTGAA | 1500 |
| GCCAGATGGG | ACAGTGCTCC | AACCAAATGA | CTTCAACTAT | ACAGATCGTT | TCTCAGTGGC | 1560 |
| CATATTCACT | GTCAACCGAG | TCTTACCTCC | TGACTCAGGA | GTCTGGGTCT | GCAGTGTGAA | 1620 |
| CACAGTGGCT | GGGATGGTGG | AAAAGCCTTT | CAACATTTCC | GTCAAAGTCC | TTCCAGAGCC | 1680 |
| CCTGCACGCC | CCAAATGTGA | TTGACACTGG | ACATAACTTT | GCTATCATCA | ATATCAGCTC | 1740 |
| TGAGCCTTAC | TTTGGGGATG | GACCCATCAA | ATCCAAGAAG | CTTTTCTATA | AACCTGTCAA | 1800 |
| TCAGGCCTGG | AAATACATTG | AAGTGACGAA | TGAGATTTTC | ACTCTCAACT | ACTTGGAGCC | 1860 |
| GCGGACTGAC | TACGAGCTGT | GTGTCCAGCT | GGCCCGTCCT | GGAGAGGGTG | GAGAAGGGCA | 1920 |
| TCCTGGGCCT | GTGAGACGAT | TTACAACAGC | GTCTATCGGA | CTCCCTCCTC | CAAGAGGTCT | 1980 |
| CAGTCTCCTG | CCAAAAGCC | AGACAGCTCT | AAATTTGACT | TGGCAACCGA | TATTTACAAA | 2040 |
| CTCAGAAGAT | GAATTTTATG | TGGAAGTCGA | GAGGCGATCC | CTGCAAACAA | CAAGTGATCA | 2100 |
| GCAGAACATC | AAAGTGCTTG | GGAACCTGAC | CTCGGTGCTA | CTGAGCAACT | TAGTCCCCAG | 2160 |
| GGAGCAGTAC | ACAGTCCGAG | CTAGAGTCAA | CACCAAGGCG | CAGGGGGAGT | GGAGTGAAGA | 2220 |

| | | | | | | |
|------------|------------|------------|------------|------------|------------|------|
| ACTCAGGGCC | TGGACCCTTA | GTGACATTCT | CCCTCCTCAA | CCAGAAAACA | TCAAGATCTC | 2280 |
| CAACATCACT | GACTCCACAG | CTATGGTTTC | TTGGACAATA | GTGGATGGCT | ATTGCGATTC | 2340 |
| TTCCATCATC | ATCCGGTATA | AGGTTCAGGG | CAAAAATGAA | GACCAGCACA | TTGATGTGAA | 2400 |
| GATCAAGAAT | GCTACCGTTA | CTCAGTACCA | GCTCAAGGGC | CTAGAGCCAG | AGACTACATA | 2460 |
| CCATGTGGAT | ATTTTTGCTG | AGAACAACAT | AGGATCAAGC | AACCCAGCCT | TTTCTCATGA | 2520 |
| ACTGAGGACG | CTTCCACATT | CCCCAGCCTC | TGCAGACCTC | GGAGGGGGAA | AGATGCTACT | 2580 |
| CATAGCCATC | CTTGGGTCGG | CTGGAATGAC | TTGCATCACC | GTGCTGTTGG | CGTTTCTGAT | 2640 |
| TATGTTGCAA | CTGAAGAGAG | CAAATGTCCA | AAGGAGAATG | GCTCAGGCAT | TCCAGAACGT | 2700 |
| GAGAGAAGAA | CCAGCTGTGC | AGTTTAACTC | AGGAACTCTG | GCCCTTAACA | GGAAGGCCAA | 2760 |
| AAACAATCCG | GATCCCACAA | TTTATCCTGT | GCTTGACTGG | AATGACATCA | AGTTTCAAGA | 2820 |
| CGTGATCGGA | GAGGGCAACT | TTGGCCAGGT | TCTGAAGGCA | CGCATCAAGA | AGGATGGGTT | 2880 |
| ACGGATGGAT | GCCGCCATCA | AGAGGATGAA | AGAGTATGCC | TCCAAAGATG | ATCACAGGGA | 2940 |
| CTTCGCAGGA | GAAGTGGAGG | TTCTTTGTAA | ACTTGACAC | CATCCAAACA | TCATCAATCT | 3000 |
| CTTGGGAGCA | TGTGAACACC | GAGGCTATTT | GTACCTAGCT | ATTGAGTATG | CCCCGCATGG | 3060 |
| AAACCTCCTG | GACTTCCTGC | GTAAGAGCAG | AGTGCTAGAG | ACAGACCCTG | CTTTTGCCAT | 3120 |
| CGCCAACAGT | ACAGCTTCCA | CACTGTCCTC | CCAACAGCTT | CTTCATTTTG | CTGCAGATGT | 3180 |
| GGCCCGGGGG | ATGGACTACT | TGAGCCAGAA | ACAGTTTATC | CACAGGGACC | TGGCTGCCAG | 3240 |
| AAACATTTTA | GTTGGTGAAA | ACTACATAGC | CAAAATAGCA | GATTTTGGAT | TGTCACGAGG | 3300 |
| TCAAGAAGTG | TATGTGAAAA | AGACAATGGG | AAGGCTCCCA | GTGCGTTGGA | TGGCAATCGA | 3360 |
| ATCACTGAAC | TATAGTGTCT | ATACAACCAA | CAGTGATGTC | TGGTCCTATG | GTGTATTGCT | 3420 |
| CTGGGAGATT | GTTAGCTTAG | GAGGCACCCC | CTACTGCGGC | ATGACGTGCG | CGGAGCTCTA | 3480 |
| TGAGAAGCTA | CCCCAGGGCT | ACAGGCTGGA | GAAGCCCCTG | AACTGTGATG | ATGAGGTGTA | 3540 |
| TGATCTAATG | AGACAGTGCT | GGAGGGAGAA | GCCTTATGAG | AGACCATCAT | TTGCCCAGAT | 3600 |
| ATTGGTGTCC | TTAAACAGGA | TGCTGGAAGA | ACGGAAGACA | TACGTGAACA | CCACACTGTA | 3660 |
| TGAGAAGTTT | ACCTATGCAG | GAATTGACTG | CTCTGCGGAA | GAAGCAGCCT | AGAGCAGAAC | 3720 |
| TCTTCATGTA | CAACGGCCAT | TTCTCCTCAC | TGGCGCGAGA | GCGCCTTGAC | ACCTGTACCA | 3780 |
| AGCAAGCCAC | CCACTGCCAA | GAGATGTGAT | ATATAAGTGT | ATATATTGTG | CTGTGTTTGG | 3840 |
| GACCCTCCTC | ATACAGCTCG | TGCGGATCTG | CAGTGTGTTT | TGACTCTAAT | GTGACTGTAT | 3900 |
| ATACTGCTCG | GAGTAAGAAT | GTGCTAAGAT | CAGAATGCCT | GTTTCGTGGT | TCATATAATA | 3960 |
| TATTTTTCTA | AAAGCATAGA | TTGCACAGGA | AGGTATGAGT | ACAAATACTG | TAATGCATAA | 4020 |
| CTTGTTACTG | TCCTAGATGT | GTTTGATATT | TTTCCTTTAC | AACTGAATGC | TATAAAAGTG | 4080 |
| TTTTGCTGTG | TACACATAAG | ATACTGTTTC | TTAAATAAAG | CATTCCCTTG | ACAGCACAGG | 4140 |
| AAGAAAAGCG | AGGGAAATGT | ATGGATTATA | TTAAATGTGG | GTTACTACAC | AAGAGGCCGA | 4200 |
| ACATTCCAAG | TAGCAGAAGA | GAGGGTCTCT | CAACTCTGCT | CCTCACCTGC | AGAAGCCAGT | 4260 |

```

TTGTTTGGCC ATGTGACAAT TGCCTGTGT TTTTATAGCA CCCAAATCAT TCTAAAATAT      4320
GAACATCTAA AAACTTTGCT AGGAGACTAA GAACCTTTGG AGAGATAGAT ATAAGTACGG      4380
TCAAAAAACA AAACGCGGG ACTTACATTT ATTTTCTATA GTAATCTGTT GTACATTTTA      4440
AGAAGTAAAA CTAGGAATTT AGGAGTGATG TGTGACATTT CTGACATGGA GTTACCATCC      4500
CCACATGTAT CACATACTGT CATATTCCCA CATGTATCAC ACATGTATTG TAAAATTTTG      4560
TAGTTTTGAT CACTTGTGAA TTTACTGTTG ATGTGGTAGC CACCTGCTGC AATGGTTCCT      4620
CTTGTAGGTG AATAAATGTC                                          4640

```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1123 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```

Met Asp Ser Leu Ala Gly Leu Val Leu Cys Gly Val Ser Leu Leu Leu
 1              5              10              15
Tyr Gly Val Val Glu Gly Ala Met Asp Leu Ile Leu Ile Asn Ser Leu
      20              25              30
Pro Leu Val Ser Asp Ala Glu Thr Ser Leu Thr Cys Ile Ala Ser Gly
      35              40              45
Trp His Pro His Glu Pro Ile Thr Ile Gly Arg Asp Phe Glu Ala Leu
      50              55              60
Met Asn Gln His Gln Asp Pro Leu Glu Val Thr Gln Asp Val Thr Arg
      65              70              75              80
Glu Trp Ala Lys Lys Val Val Trp Lys Arg Glu Lys Ala Ser Lys Ile
      85              90              95
Asn Gly Ala Tyr Phe Cys Glu Gly Arg Val Arg Gly Gln Ala Ile Arg
      100              105              110
Ile Arg Thr Met Lys Met Arg Gln Gln Ala Ser Phe Leu Pro Ala Thr
      115              120              125
Leu Thr Met Thr Val Asp Arg Gly Asp Asn Val Asn Ile Ser Phe Lys
      130              135              140
Lys Val Leu Ile Lys Glu Glu Asp Ala Val Ile Tyr Lys Asn Gly Ser
      145              150              155              160
Phe Ile His Ser Val Pro Arg His Glu Val Pro Asp Ile Leu Glu Val
      165              170              175
His Leu Pro His Ala Gln Pro Gln Asp Ala Gly Val Tyr Ser Ala Arg
      180              185              190
Tyr Ile Gly Gly Asn Leu Phe Thr Ser Ala Phe Thr Arg Leu Ile Val

```

| 195 | | | | | 200 | | | | | 205 | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Arg | Arg | Cys | Glu | Ala | Gln | Lys | Trp | Gly | Pro | Asp | Cys | Ser | Arg | Pro | Cys |
| 210 | | | | | | 215 | | | | | 220 | | | | |
| Thr | Thr | Cys | Lys | Asn | Asn | Gly | Val | Cys | His | Glu | Asp | Thr | Gly | Glu | Cys |
| 225 | | | | 230 | | | | | | 235 | | | | | 240 |
| Ile | Cys | Pro | Pro | Gly | Phe | Met | Gly | Arg | Thr | Cys | Glu | Lys | Ala | Cys | Glu |
| | | | | 245 | | | | | 250 | | | | | 255 | |
| Pro | His | Thr | Phe | Gly | Arg | Thr | Cys | Lys | Glu | Arg | Cys | Ser | Gly | Pro | Glu |
| | | | 260 | | | | | 265 | | | | | 270 | | |
| Gly | Cys | Lys | Ser | Tyr | Val | Phe | Cys | Leu | Pro | Asp | Pro | Tyr | Gly | Cys | Ser |
| | | 275 | | | | | 280 | | | | | 285 | | | |
| Cys | Ala | Thr | Gly | Trp | Arg | Gly | Leu | Gln | Cys | Asn | Glu | Ala | Cys | Pro | Ser |
| | 290 | | | | | 295 | | | | | 300 | | | | |
| Gly | Tyr | Tyr | Gly | Pro | Asp | Cys | Lys | Leu | Arg | Cys | His | Cys | Thr | Asn | Glu |
| 305 | | | | 310 | | | | | | 315 | | | | | 320 |
| Glu | Ile | Cys | Asp | Arg | Phe | Gln | Gly | Cys | Leu | Cys | Ser | Gln | Gly | Trp | Gln |
| | | | 325 | | | | | | 330 | | | | | 335 | |
| Gly | Leu | Gln | Cys | Glu | Lys | Glu | Gly | Arg | Pro | Arg | Met | Thr | Pro | Gln | Ile |
| | | | 340 | | | | | 345 | | | | | 350 | | |
| Glu | Asp | Leu | Pro | Asp | His | Ile | Glu | Val | Asn | Ser | Gly | Lys | Phe | Asn | Pro |
| | | 355 | | | | | 360 | | | | | 365 | | | |
| Ile | Cys | Lys | Ala | Ser | Gly | Trp | Pro | Leu | Pro | Thr | Ser | Glu | Glu | Met | Thr |
| | 370 | | | | | 375 | | | | | 380 | | | | |
| Leu | Val | Lys | Pro | Asp | Gly | Thr | Val | Leu | Gln | Pro | Asn | Asp | Phe | Asn | Tyr |
| 385 | | | | 390 | | | | | | 395 | | | | | 400 |
| Thr | Asp | Arg | Phe | Ser | Val | Ala | Ile | Phe | Thr | Val | Asn | Arg | Val | Leu | Pro |
| | | | 405 | | | | | | 410 | | | | | 415 | |
| Pro | Asp | Ser | Gly | Val | Trp | Val | Cys | Ser | Val | Asn | Thr | Val | Ala | Gly | Met |
| | | | 420 | | | | | 425 | | | | | 430 | | |
| Val | Glu | Lys | Pro | Phe | Asn | Ile | Ser | Val | Lys | Val | Leu | Pro | Glu | Pro | Leu |
| | | 435 | | | | | 440 | | | | | 445 | | | |
| His | Ala | Pro | Asn | Val | Ile | Asp | Thr | Gly | His | Asn | Phe | Ala | Ile | Ile | Asn |
| | 450 | | | | | 455 | | | | | 460 | | | | |
| Ile | Ser | Ser | Glu | Pro | Tyr | Phe | Gly | Asp | Gly | Pro | Ile | Lys | Ser | Lys | Lys |
| 465 | | | | 470 | | | | | | 475 | | | | | 480 |
| Leu | Phe | Tyr | Lys | Pro | Val | Asn | Gln | Ala | Trp | Lys | Tyr | Ile | Glu | Val | Thr |
| | | | 485 | | | | | | 490 | | | | | 495 | |
| Asn | Glu | Ile | Phe | Thr | Leu | Asn | Tyr | Leu | Glu | Pro | Arg | Thr | Asp | Tyr | Glu |
| | | | 500 | | | | | 505 | | | | | 510 | | |
| Leu | Cys | Val | Gln | Leu | Ala | Arg | Pro | Gly | Glu | Gly | Gly | Glu | Gly | His | Pro |
| | | 515 | | | | | 520 | | | | | 525 | | | |
| Gly | Pro | Val | Arg | Arg | Phe | Thr | Thr | Ala | Ser | Ile | Gly | Leu | Pro | Pro | Pro |
| | 530 | | | | | 535 | | | | | 540 | | | | |
| Arg | Gly | Leu | Ser | Leu | Leu | Pro | Lys | Ser | Gln | Thr | Ala | Leu | Asn | Leu | Thr |
| 545 | | | | 550 | | | | | | 555 | | | | | 560 |

Trp Gln Pro Ile Phe Thr Asn Ser Glu Asp Glu Phe Tyr Val Glu Val
 565 570 575
 Glu Arg Arg Ser Leu Gln Thr Thr Ser Asp Gln Gln Asn Ile Lys Val
 580 585 590
 Pro Gly Asn Leu Thr Ser Val Leu Leu Ser Asn Leu Val Pro Arg Glu
 595 600 605
 Gln Tyr Thr Val Arg Ala Arg Val Asn Thr Lys Ala Gln Gly Glu Trp
 610 615 620
 Ser Glu Glu Leu Arg Ala Trp Thr Leu Ser Asp Ile Leu Pro Pro Gln
 625 630 635 640
 Pro Glu Asn Ile Lys Ile Ser Asn Ile Thr Asp Ser Thr Ala Met Val
 645 650 655
 Ser Trp Thr Ile Val Asp Gly Tyr Ser Ile Ser Ser Ile Ile Ile Arg
 660 665 670
 Tyr Lys Val Gln Gly Lys Asn Glu Asp Gln His Ile Asp Val Lys Ile
 675 680 685
 Lys Asn Ala Thr Val Thr Gln Tyr Gln Leu Lys Gly Leu Glu Pro Glu
 690 695 700
 Thr Thr Tyr His Val Asp Ile Phe Ala Glu Asn Asn Ile Gly Ser Ser
 705 710 715 720
 Asn Pro Ala Phe Ser His Glu Leu Arg Thr Leu Pro His Ser Pro Ala
 725 730 735
 Ser Ala Asp Leu Gly Gly Gly Lys Met Leu Leu Ile Ala Ile Leu Gly
 740 745 750
 Ser Ala Gly Met Thr Cys Ile Thr Val Leu Leu Ala Phe Leu Ile Met
 755 760 765
 Leu Gln Leu Lys Arg Ala Asn Val Gln Arg Arg Met Ala Gln Ala Phe
 770 775 780
 Gln Asn Val Arg Glu Glu Pro Ala Val Gln Phe Asn Ser Gly Thr Leu
 785 790 795 800
 Ala Leu Asn Arg Lys Ala Lys Asn Asn Pro Asp Pro Thr Ile Tyr Pro
 805 810 815
 Val Leu Asp Trp Asn Asp Ile Lys Phe Gln Asp Val Ile Gly Glu Gly
 820 825 830
 Asn Phe Gly Gln Val Leu Lys Ala Arg Ile Lys Lys Asp Gly Leu Arg
 835 840 845
 Met Asp Ala Ala Ile Lys Arg Met Lys Glu Tyr Ala Ser Lys Asp Asp
 850 855 860
 His Arg Asp Phe Ala Gly Glu Leu Glu Val Leu Cys Lys Leu Gly His
 865 870 875 880
 His Pro Asn Ile Ile Asn Leu Leu Gly Ala Cys Glu His Arg Gly Tyr
 885 890 895
 Leu Tyr Leu Ala Ile Glu Tyr Ala Pro His Gly Asn Leu Leu Asp Phe
 900 905 910
 Leu Arg Lys Ser Arg Val Leu Glu Thr Asp Pro Ala Phe Ala Ile Ala

| 915 | | | | | 920 | | | | | 925 | | | | | |
|------|------|------|------|------|------|------|------|------|------|------|------|------|-----|------|------|
| Asn | Ser | Thr | Ala | Ser | Thr | Leu | Ser | Ser | Gln | Gln | Leu | Leu | His | Phe | Ala |
| 930 | | | | | | 935 | | | | | 940 | | | | |
| Ala | Asp | Val | Ala | Arg | Gly | Met | Asp | Tyr | Leu | Ser | Gln | Lys | Gln | Phe | Ile |
| 945 | | | | | 950 | | | | | 955 | | | | | 960 |
| His | Arg | Asp | Leu | Ala | Ala | Arg | Asn | Ile | Leu | Val | Gly | Glu | Asn | Tyr | Ile |
| | | | | 965 | | | | | 970 | | | | | 975 | |
| Ala | Lys | Ile | Ala | Asp | Phe | Gly | Leu | Ser | Arg | Gly | Gln | Glu | Val | Tyr | Val |
| | | | 980 | | | | | 985 | | | | | 990 | | |
| Lys | Lys | Thr | Met | Gly | Arg | Leu | Pro | Val | Arg | Trp | Met | Ala | Ile | Glu | Ser |
| | | 995 | | | | | 1000 | | | | | 1005 | | | |
| Leu | Asn | Tyr | Ser | Val | Tyr | Thr | Thr | Asn | Ser | Asp | Val | Trp | Ser | Tyr | Gly |
| | 1010 | | | | | 1015 | | | | | 1020 | | | | |
| Val | Leu | Leu | Trp | Glu | Ile | Val | Ser | Leu | Gly | Gly | Thr | Pro | Tyr | Cys | Gly |
| 1025 | | | | | 1030 | | | | | 1035 | | | | | 1040 |
| Met | Thr | Cys | Ala | Glu | Leu | Tyr | Glu | Lys | Leu | Pro | Gln | Gly | Tyr | Arg | Leu |
| | | | | 1045 | | | | | 1050 | | | | | 1055 | |
| Glu | Lys | Pro | Leu | Asn | Cys | Asp | Asp | Glu | Val | Tyr | Asp | Leu | Met | Arg | Gln |
| | | | 1060 | | | | | 1065 | | | | 1070 | | | |
| Cys | Trp | Arg | Glu | Lys | Pro | Tyr | Glu | Arg | Pro | Ser | Phe | Ala | Gln | Ile | Leu |
| | | 1075 | | | | | 1080 | | | | | 1085 | | | |
| Val | Ser | Leu | Asn | Arg | Met | Leu | Glu | Glu | Arg | Lys | Thr | Tyr | Val | Asn | Thr |
| | 1090 | | | | | 1095 | | | | | 1100 | | | | |
| Thr | Leu | Tyr | Glu | Lys | Phe | Thr | Tyr | Ala | Gly | Ile | Asp | Cys | Ser | Ala | Glu |
| 1105 | | | | | 1110 | | | | | 1115 | | | | | 1120 |
| Glu | Ala | Ala | | | | | | | | | | | | | |

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 301 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ile | Lys | Phe | Gln | Asp | Val | Ile | Gly | Glu | Gly | Asn | Phe | Gly | Gln | Val | Leu |
| 1 | | | | 5 | | | | | 10 | | | | | 15 | |
| Lys | Ala | Arg | Ile | Lys | Lys | Asp | Gly | Leu | Arg | Met | Asp | Ala | Ala | Ile | Lys |
| | | | 20 | | | | | 25 | | | | | 30 | | |
| Arg | Met | Lys | Glu | Tyr | Ala | Ser | Lys | Asp | Asp | His | Arg | Asp | Phe | Ala | Gly |
| | | 35 | | | | | 40 | | | | | 45 | | | |
| Glu | Leu | Glu | Val | Leu | Cys | Lys | Leu | Gly | His | His | Pro | Asn | Ile | Ile | Asn |

| | | |
|---------|-----------------------------|-----------------------------|
| 50 | 55 | 60 |
| Leu 65 | Leu Gly Ala Cys 70 | Glu His Arg Gly Tyr 75 |
| Tyr 85 | Ala Pro Met Gly 85 | Asn Leu Leu Asp Phe 90 |
| Leu 100 | Glu Thr Asp Pro Ala Phe 100 | Ala Ile Ala Asn Ser Thr 110 |
| Leu 115 | Ser Ser Gln Gln Leu Leu 115 | His Phe Ala Ala Asp Val 125 |
| Met 130 | Asp Tyr Leu Ser Gln Lys 135 | Gln Phe Ile His Arg Asp 140 |
| Arg 145 | Asn Ile Leu Val Gly 150 | Glu Asn Tyr Ile Ala Lys 155 |
| Gly 165 | Leu Ser Arg Gly 165 | Gln Glu Val Tyr Val 170 |
| Leu 180 | Pro Val Arg Trp Met Ala 180 | Ile Glu Ser Leu Asn Tyr 190 |
| Thr 195 | Thr Asn Ser Asp Val Trp 195 | Ser Tyr Gly Val Leu 205 |
| Val 210 | Ser Leu Gly Gly Thr 210 | Pro Tyr Cys Gly Met 220 |
| Tyr 225 | Glu Lys Leu Pro Gln 230 | Gly Tyr Arg Leu Glu 235 |
| Asp 245 | Asp Glu Val Tyr 245 | Asp Leu Met Arg Gln 250 |
| Tyr 260 | Glu Arg Pro Ser Phe 260 | Ala Gln Ile Leu Val 270 |
| Leu 275 | Glu Glu Arg Lys Thr 275 | Tyr Val Asn Thr Thr 285 |
| Thr 290 | Tyr Ala Gly Ile Asp 295 | Cys Ser Ala Glu Glu 300 |

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1124 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: protein
- (iii) HYPOTHETICAL: NO
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

| | | | | | | | | | | | | | | |
|--------|--------|--------|--------|--------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| Met 1 | Asp 5 | Ser 10 | Leu 15 | Ala 20 | Ser 25 | Leu 30 | Val 35 | Leu 40 | Cys 45 | Gly 50 | Val 55 | Ser 60 | Leu 65 | Leu 70 |
| Ser 75 | Gly 80 | Thr 85 | Val 90 | Glu 95 | Gly 100 | Ala 105 | Met 110 | Asp 115 | Leu 120 | Ile 125 | Leu 130 | Ile 135 | Asn 140 | Ser 145 |

| 20 | | | | | 25 | | | | | 30 | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Pro | Leu | Val | Ser | Asp | Ala | Glu | Thr | Ser | Leu | Thr | Cys | Ile | Ala | Ser | Gly |
| | | 35 | | | | | 40 | | | | | 45 | | | |
| Trp | Arg | Pro | His | Glu | Pro | Ile | Thr | Ile | Gly | Arg | Asp | Phe | Glu | Ala | Leu |
| | 50 | | | | | 55 | | | | | 60 | | | | |
| Met | Asn | Gln | His | Gln | Asp | Pro | Leu | Glu | Val | Thr | Gln | Asp | Val | Thr | Arg |
| | 65 | | | | 70 | | | | | 75 | | | | | 80 |
| Glu | Trp | Ala | Lys | Lys | Val | Val | Trp | Lys | Arg | Glu | Lys | Ala | Ser | Lys | Ile |
| | | | | 85 | | | | | 90 | | | | | 95 | |
| Asn | Gly | Ala | Tyr | Phe | Cys | Glu | Gly | Arg | Val | Arg | Gly | Glu | Ala | Ile | Arg |
| | | | 100 | | | | | 105 | | | | | 110 | | |
| Ile | Arg | Thr | Met | Lys | Met | Arg | Gln | Gln | Ala | Ser | Phe | Leu | Pro | Ala | Thr |
| | | 115 | | | | | 120 | | | | | 125 | | | |
| Leu | Thr | Met | Thr | Val | Asp | Lys | Gly | Asp | Asn | Val | Asn | Ile | Ser | Phe | Lys |
| | 130 | | | | | 135 | | | | | 140 | | | | |
| Lys | Val | Leu | Ile | Lys | Glu | Glu | Asp | Ala | Val | Ile | Tyr | Lys | Asn | Gly | Ser |
| | 145 | | | | 150 | | | | | 155 | | | | | 160 |
| Phe | Ile | His | Ser | Val | Pro | Arg | His | Glu | Val | Pro | Asp | Ile | Leu | Glu | Val |
| | | | | 165 | | | | | 170 | | | | | 175 | |
| His | Leu | Pro | His | Ala | Gln | Pro | Gln | Asp | Ala | Gly | Val | Tyr | Ser | Ala | Arg |
| | | | 180 | | | | | 185 | | | | | 190 | | |
| Tyr | Ile | Gly | Gly | Asn | Leu | Phe | Thr | Ser | Ala | Phe | Thr | Arg | Leu | Ile | Val |
| | | 195 | | | | | 200 | | | | | 205 | | | |
| Arg | Arg | Cys | Glu | Ala | Gln | Lys | Trp | Gly | Pro | Glu | Cys | Asn | His | Leu | Cys |
| | 210 | | | | | 215 | | | | | 220 | | | | |
| Thr | Ala | Cys | Met | Asn | Asn | Gly | Val | Cys | His | Glu | Asp | Thr | Gly | Glu | Cys |
| | 225 | | | | 230 | | | | | 235 | | | | | 240 |
| Ile | Cys | Pro | Pro | Gly | Phe | Met | Gly | Arg | Thr | Cys | Glu | Lys | Ala | Cys | Glu |
| | | | | 245 | | | | | 250 | | | | | 255 | |
| Leu | His | Thr | Phe | Gly | Arg | Thr | Cys | Lys | Glu | Arg | Cys | Ser | Gly | Gln | Glu |
| | | | 260 | | | | | 265 | | | | | 270 | | |
| Gly | Cys | Lys | Ser | Tyr | Val | Phe | Cys | Leu | Pro | Asp | Pro | Tyr | Gly | Cys | Ser |
| | | 275 | | | | | 280 | | | | | 285 | | | |
| Cys | Ala | Thr | Gly | Trp | Lys | Gly | Leu | Gln | Cys | Asn | Glu | Ala | Cys | His | Pro |
| | 290 | | | | | 295 | | | | | 300 | | | | |
| Gly | Phe | Tyr | Gly | Pro | Asp | Cys | Lys | Leu | Arg | Cys | Ser | Cys | Asn | Asn | Gly |
| | 305 | | | | 310 | | | | | 315 | | | | | 320 |
| Glu | Met | Cys | Asp | Arg | Phe | Gln | Gly | Cys | Leu | Cys | Ser | Pro | Gly | Trp | Gln |
| | | | | 325 | | | | | 330 | | | | | 335 | |
| Gly | Leu | Gln | Cys | Glu | Arg | Glu | Gly | Ile | Pro | Arg | Met | Thr | Pro | Lys | Ile |
| | | | 340 | | | | | 345 | | | | | 350 | | |
| Val | Asp | Leu | Pro | Asp | His | Ile | Glu | Val | Asn | Ser | Gly | Lys | Phe | Asn | Pro |
| | | 355 | | | | | 360 | | | | | 365 | | | |
| Ile | Cys | Lys | Ala | Ser | Gly | Trp | Pro | Leu | Pro | Thr | Asn | Glu | Glu | Met | Thr |
| | 370 | | | | | 375 | | | | | 380 | | | | |

Leu Val Lys Pro Asp Gly Thr Val Leu His Pro Lys Asp Phe Asn His
 385 390 395 400
 Thr Asp His Phe Ser Val Ala Il Phe Thr Ile His Arg Ile Leu Pro
 405 410 415
 Pro Asp Ser Gly Val Trp Val Cys Ser Val Asn Thr Val Ala Gly Met
 420 425 430
 Val Glu Lys Pro Phe Asn Ile Ser Val Lys Val Leu Pro Lys Pro Leu
 435 440 445
 Asn Ala Pro Asn Val Ile Asp Thr Gly His Asn Phe Ala Val Ile Asn
 450 455 460
 Ile Ser Ser Glu Pro Tyr Phe Gly Asp Gly Pro Ile Lys Ser Lys Lys
 465 470 475 480
 Leu Leu Tyr Lys Pro Val Asn His Tyr Glu Ala Trp Gln His Ile Gln
 485 490 495
 Val Thr Asn Glu Ile Val Thr Leu Asn Tyr Leu Glu Pro Arg Thr Glu
 500 505 510
 Tyr Glu Leu Cys Val Gln Leu Val Arg Arg Gly Glu Gly Gly Glu Gly
 515 520 525
 His Pro Gly Pro Val Arg Arg Phe Thr Thr Ala Ser Ile Gly Leu Pro
 530 535 540
 Pro Pro Arg Gly Leu Asn Leu Leu Pro Lys Ser Gln Thr Thr Leu Asn
 545 550 555 560
 Leu Thr Trp Gln Pro Ile Phe Pro Ser Ser Glu Asp Asp Phe Tyr Val
 565 570 575
 Glu Val Glu Arg Arg Ser Val Gln Lys Ser Asp Gln Gln Asn Ile Lys
 580 585 590
 Val Pro Gly Asn Leu Thr Ser Val Leu Leu Asn Asn Leu His Pro Arg
 595 600 605
 Glu Gln Tyr Val Val Arg Ala Arg Val Asn Thr Lys Ala Gln Gly Glu
 610 615 620
 Trp Ser Glu Asp Leu Thr Ala Trp Thr Leu Ser Asp Ile Leu Pro Pro
 625 630 635 640
 Gln Pro Glu Asn Ile Lys Ile Ser Asn Ile Thr His Ser Ser Ala Val
 645 650 655
 Ile Ser Trp Thr Ile Leu Asp Gly Tyr Ser Ile Ser Ser Ile Thr Ile
 660 665 670
 Arg Tyr Lys Val Gln Gly Lys Asn Glu Asp Gln His Val Asp Val Lys
 675 680 685
 Ile Lys Asn Ala Thr Ile Ile Gln Tyr Gln Leu Lys Gly Leu Glu Pro
 690 695 700
 Glu Thr Ala Tyr Gln Val Asp Ile Phe Ala Glu Asn Asn Ile Gly Ser
 705 710 715 720
 Ser Asn Pro Ala Phe Ser His Glu Leu Val Thr Leu Pro Glu Ser Gln
 725 730 735
 Ala Pro Ala Asp Leu Gly Gly Gly Lys Met Leu Leu Ile Ala Ile Leu

| 740 | | | | | 745 | | | | | 750 | | | | | |
|------|------|------|------|-----|------|------|------|------|------|------|------|------|------|------|------|
| Gly | Ser | Ala | Gly | Met | Thr | Cys | Leu | Thr | Val | Leu | Leu | Ala | Phe | Leu | Ile |
| | | 755 | | | | | 760 | | | | | 765 | | | |
| Ile | Leu | Gln | Leu | Lys | Arg | Ala | Asn | Val | Gln | Arg | Arg | Met | Ala | Gln | Ala |
| | 770 | | | | | 775 | | | | | 780 | | | | |
| Phe | Gln | Asn | Val | Arg | Glu | Glu | Pro | Ala | Val | Gln | Phe | Asn | Ser | Gly | Thr |
| 785 | | | | | 790 | | | | | 795 | | | | | 800 |
| Leu | Ala | Leu | Asn | Arg | Lys | Val | Lys | Asn | Asn | Pro | Asp | Pro | Thr | Ile | Tyr |
| | | | 805 | | | | | | 810 | | | | | 815 | |
| Pro | Val | Leu | Asp | Trp | Asn | Asp | Ile | Lys | Phe | Gln | Asp | Val | Ile | Gly | Glu |
| | | | 820 | | | | | 825 | | | | | 830 | | |
| Gly | Asn | Phe | Gly | Gln | Val | Leu | Lys | Ala | Arg | Ile | Lys | Lys | Asp | Gly | Leu |
| | | 835 | | | | | 840 | | | | | 845 | | | |
| Arg | Met | Asp | Ala | Ala | Ile | Lys | Arg | Met | Lys | Glu | Tyr | Ala | Ser | Lys | Asp |
| | 850 | | | | | 855 | | | | | 860 | | | | |
| Asp | His | Arg | Asp | Phe | Ala | Gly | Glu | Leu | Glu | Val | Leu | Cys | Lys | Leu | Gly |
| 865 | | | | | 870 | | | | | 875 | | | | | 880 |
| His | His | Pro | Asn | Ile | Ile | Asn | Leu | Leu | Gly | Ala | Cys | Glu | His | Arg | Gly |
| | | | 885 | | | | | | 890 | | | | | 895 | |
| Tyr | Leu | Tyr | Leu | Ala | Ile | Glu | Tyr | Ala | Pro | His | Gly | Asn | Leu | Leu | Asp |
| | | | 900 | | | | | 905 | | | | | 910 | | |
| Phe | Leu | Arg | Lys | Ser | Arg | Val | Leu | Glu | Thr | Asp | Pro | Ala | Phe | Ala | Ile |
| | | 915 | | | | | 920 | | | | | 925 | | | |
| Ala | Asn | Ser | Thr | Ala | Ser | Thr | Leu | Ser | Ser | Gln | Gln | Leu | Leu | His | Phe |
| | 930 | | | | | 935 | | | | | 940 | | | | |
| Ala | Ala | Asp | Val | Ala | Arg | Gly | Met | Asp | Tyr | Leu | Ser | Gln | Lys | Gln | Phe |
| 945 | | | | | 950 | | | | | 955 | | | | | 960 |
| Ile | His | Arg | Asp | Leu | Ala | Ala | Arg | Asn | Ile | Leu | Val | Gly | Glu | Asn | Tyr |
| | | | 965 | | | | | 970 | | | | | | 975 | |
| Val | Ala | Lys | Ile | Ala | Asp | Phe | Gly | Leu | Ser | Arg | Gly | Gln | Glu | Val | Tyr |
| | | | 980 | | | | | 985 | | | | | 990 | | |
| Val | Lys | Lys | Thr | Met | Gly | Arg | Leu | Pro | Val | Arg | Trp | Met | Ala | Ile | Glu |
| | | 995 | | | | | 1000 | | | | | 1005 | | | |
| Ser | Leu | Asn | Tyr | Ser | Val | Tyr | Thr | Thr | Asn | Ser | Asp | Val | Trp | Ser | Tyr |
| | 1010 | | | | | 1015 | | | | | 1020 | | | | |
| Gly | Val | Leu | Leu | Trp | Glu | Ile | Val | Ser | Leu | Gly | Gly | Thr | Pro | Tyr | Cys |
| 1025 | | | | | 1030 | | | | | 1035 | | | | | 1040 |
| Gly | Met | Thr | Cys | Ala | Glu | Leu | Tyr | Glu | Lys | Leu | Pro | Gln | Gly | Tyr | Arg |
| | | | 1045 | | | | | | 1050 | | | | | 1055 | |
| Leu | Glu | Lys | Pro | Leu | Asn | Cys | Asp | Asp | Glu | Val | Tyr | Asp | Leu | Met | Arg |
| | | | 1060 | | | | | 1065 | | | | | 1070 | | |
| Gln | Cys | Trp | Arg | Glu | Lys | Pro | Tyr | Glu | Arg | Pro | Ser | Phe | Ala | Gln | Ile |
| | | 1075 | | | | | 1080 | | | | | 1085 | | | |
| Leu | Val | Ser | Leu | Asn | Arg | Met | Leu | Glu | Glu | Arg | Lys | Thr | Tyr | Val | Asn |
| | 1090 | | | | | 1095 | | | | | 1100 | | | | |

Thr Thr Leu Tyr Glu Lys Phe Thr Tyr Ala Gly Ile Asp Cys Ser Ala
 1105 1110 1115 1120
 Glu Glu Ala Ala

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 1138 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Met | Val | Trp | Arg | Val | Pro | Pro | Phe | Leu | Leu | Pro | Ile | Leu | Phe | Leu | Ala |
| 1 | | | | 5 | | | | | 10 | | | | | 15 | |
| Ser | His | Val | Gly | Ala | Ala | Val | Asp | Leu | Thr | Leu | Leu | Ala | Asn | Leu | Arg |
| | | | 20 | | | | | 25 | | | | | 30 | | |
| Leu | Thr | Asp | Pro | Gln | Arg | Phe | Phe | Leu | Thr | Cys | Val | Ser | Gly | Glu | Ala |
| | | 35 | | | | | 40 | | | | | 45 | | | |
| Gly | Ala | Gly | Arg | Gly | Ser | Asp | Ala | Trp | Gly | Pro | Pro | Leu | Leu | Leu | Glu |
| | 50 | | | | | 55 | | | | 60 | | | | | |
| Lys | Asp | Asp | Arg | Ile | Val | Arg | Thr | Pro | Pro | Gly | Pro | Pro | Leu | Arg | Leu |
| 65 | | | | 70 | | | | | | 75 | | | | 80 | |
| Ala | Arg | Asn | Gly | Ser | His | Gln | Val | Thr | Leu | Arg | Gly | Phe | Ser | Lys | Pro |
| | | | 85 | | | | | | 90 | | | | 95 | | |
| Ser | Asp | Leu | Val | Gly | Val | Phe | Ser | Cys | Val | Gly | Gly | Ala | Gly | Ala | Arg |
| | | | 100 | | | | | 105 | | | | | 110 | | |
| Arg | Thr | Arg | Val | Ile | Tyr | Val | His | Asn | Ser | Pro | Gly | Ala | His | Leu | Leu |
| | | | 115 | | | | 120 | | | | | 125 | | | |
| Pro | Asp | Lys | Val | Thr | His | Thr | Val | Asn | Lys | Gly | Asp | Thr | Ala | Val | Leu |
| | 130 | | | | | 135 | | | | | 140 | | | | |
| Ser | Ala | Arg | Val | His | Lys | Glu | Lys | Gln | Thr | Asp | Val | Ile | Trp | Lys | Ser |
| 145 | | | | 150 | | | | | | 155 | | | | 160 | |
| Asn | Gly | Ser | Tyr | Phe | Tyr | Thr | Leu | Asp | Trp | His | Glu | Ala | Gln | Asp | Gly |
| | | | 165 | | | | | | 170 | | | | 175 | | |
| Arg | Phe | Leu | Leu | Gln | Leu | Pro | Asn | Val | Gln | Pro | Pro | Ser | Ser | Gly | Ile |
| | | | 180 | | | | | 185 | | | | | 190 | | |
| Tyr | Ser | Ala | Thr | Tyr | Leu | Glu | Ala | Ser | Pro | Leu | Gly | Ser | Ala | Phe | Phe |
| | | 195 | | | | | 200 | | | | | 205 | | | |
| Arg | Leu | Ile | Val | Arg | Gly | Cys | Gly | Ala | Gly | Arg | Trp | Gly | Pro | Gly | Cys |
| | 210 | | | | | 215 | | | | | 220 | | | | |
| Thr | Lys | Glu | Cys | Pro | Gly | Cys | Leu | His | Gly | Gly | Val | Cys | His | Asp | His |
| 225 | | | | | 230 | | | | | 235 | | | | 240 | |

Asp Gly Glu Cys Val Cys Pro Pro Gly Phe Thr Gly Thr Arg Cys Glu
 245 250 255
 Gln Ala Cys Arg Glu Gly Arg Phe Gly Gln Ser Cys Gln Glu Gln Cys
 260 265 270
 Pro Gly Ile Ser Gly Cys Arg Gly Leu Thr Phe Cys Leu Pro Asp Pro
 275 280 285
 Tyr Gly Cys Ser Cys Gly Ser Gly Trp Arg Gly Ser Gln Cys Gln Glu
 290 295 300
 Ala Cys Ala Pro Gly His Phe Gly Ala Asp Cys Arg Leu Gln Cys Gln
 305 310 315 320
 Cys Gln Asn Gly Gly Thr Cys Asp Arg Phe Ser Gly Cys Val Cys Pro
 325 330 335
 Ser Gly Trp His Gly Val His Cys Glu Lys Ser Asp Arg Ile Pro Gln
 340 345 350
 Ile Leu Asn Met Ala Ser Glu Leu Glu Phe Asn Leu Glu Thr Met Pro
 355 360 365
 Arg Ile Asn Cys Ala Ala Ala Gly Asn Pro Phe Pro Val Arg Gly Ser
 370 375 380
 Ile Glu Leu Arg Lys Pro Asp Gly Thr Val Leu Leu Ser Thr Lys Ala
 385 390 395 400
 Ile Val Glu Pro Glu Lys Thr Thr Ala Glu Phe Glu Val Pro Arg Leu
 405 410 415
 Val Leu Ala Asp Ser Gly Phe Trp Glu Cys Arg Val Ser Thr Ser Gly
 420 425 430
 Gly Gln Asp Ser Arg Arg Phe Lys Val Asn Val Lys Val Pro Pro Val
 435 440 445
 Pro Leu Ala Ala Pro Arg Leu Leu Thr Lys Gln Ser Arg Gln Leu Val
 450 455 460
 Val Ser Pro Leu Val Ser Phe Ser Gly Asp Gly Pro Ile Ser Thr Val
 465 470 475 480
 Arg Leu His Tyr Arg Pro Gln Asp Ser Thr Met Asp Trp Ser Thr Ile
 485 490 495
 Val Val Asp Pro Ser Glu Asn Val Thr Leu Met Asn Leu Arg Pro Lys
 500 505 510
 Thr Gly Tyr Ser Val Arg Val Gln Leu Ser Arg Pro Gly Glu Gly Gly
 515 520 525
 Glu Gly Ala Trp Gly Pro Pro Thr Leu Met Thr Thr Asp Cys Pro Glu
 530 535 540
 Pro Leu Leu Gln Pro Trp Leu Glu Gly Trp His Val Glu Gly Thr Asp
 545 550 555 560
 Arg Leu Arg Val Ser Trp Ser Leu Pro Leu Val Pro Gly Pro Leu Val
 565 570 575
 Gly Asp Gly Phe Leu Leu Arg Leu Trp Asp Gly Thr Arg Gly Gln Glu
 580 585 590
 Arg Arg Glu Asn Val Ser Ser Pro Gln Ala Arg Thr Ala Leu Leu Thr

| 595 | | | | | 600 | | | | | 605 | | | | | |
|---------|---------|-----|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|-----|---------|
| Gly 610 | Leu | Thr | Pro | Gly | Thr | His 615 | Tyr | Gln | Leu | Asp | Val 620 | Gln | Leu | Tyr | His |
| Cys 625 | Thr | Leu | Leu | Gly | Pro 630 | Ala | Ser | Pro | Pro | Ala 635 | His | Val | Leu | Leu | Pro 640 |
| Pro | Ser | Gly | Pro | Pro 645 | Ala | Pro | Arg | His | Leu 650 | His | Ala | Gln | Ala | Leu | Ser 655 |
| Asp | Ser | Glu | Ile 660 | Gln | Leu | Thr | Trp | Lys 665 | His | Pro | Glu | Ala | Leu 670 | Pro | Gly |
| Pro | Ile | Ser | Lys 675 | Tyr | Val | Val | Glu 680 | Val | Gln | Val | Ala | Gly 685 | Gly | Ala | Gly |
| Asp | Pro | Leu | Trp | Ile | Asp 690 | Val | Asp 695 | Arg | Pro | Glu | Glu 700 | Thr | Ser | Thr | Ile |
| Ile 705 | Arg | Gly | Leu | Asn 710 | Ala | Ser | Thr | Arg | Tyr | Leu 715 | Phe | Arg | Met | Arg | Ala 720 |
| Ser | Ile | Gln | Gly 725 | Leu | Gly | Asp | Trp | Ser | Asn 730 | Thr | Val | Glu | Glu | Ser | Thr 735 |
| Leu | Gly | Asn | Gly 740 | Leu | Gln | Ala | Glu | Gly 745 | Pro | Val | Gln | Glu | Ser | Arg | Ala 750 |
| Ala | Glu | Glu | Gly 755 | Leu | Asp | Gln | Gln | Leu 760 | Ile | Leu | Ala | Val 765 | Val | Gly | Ser |
| Val | Ser | Ala | Thr | Cys | Leu 770 | Thr 775 | Ile | Leu | Ala | Ala | Leu 780 | Leu | Thr | Leu | Val |
| Cys 785 | Ile | Arg | Arg | Ser | Cys 790 | Leu | His | Arg | Arg | Arg 795 | Thr | Phe | Thr | Tyr | Gln 800 |
| Ser | Gly | Ser | Gly 805 | Glu | Glu | Thr | Ile | Leu | Gln 810 | Phe | Ser | Ser | Gly | Thr | Leu 815 |
| Thr | Leu | Thr | Arg 820 | Arg | Pro | Lys | Leu | Gln 825 | Pro | Glu | Pro | Leu | Ser | Tyr | Pro 830 |
| Val | Leu | Glu | Trp 835 | Glu | Asp | Ile | Thr 840 | Phe | Glu | Asp | Leu | Ile 845 | Gly | Glu | Gly |
| Asn 850 | Phe | Gly | Gln | Val | Ile 855 | Arg | Ala | Met | Ile | Lys | Lys 860 | Asp | Gly | Leu | Lys |
| Met 865 | Asn | Ala | Ala | Ile | Lys 870 | Met | Leu | Lys | Glu | Tyr 875 | Ala | Ser | Glu | Asn | Asp 880 |
| His | Arg | Asp | Phe 885 | Ala | Gly | Glu | Leu | Glu | Val 890 | Leu | Cys | Lys | Leu | Gly | His 895 |
| His | Pro | Asn | Ile 900 | Ile | Asn | Leu | Leu | Gly 905 | Ala | Cys | Lys | Asn 910 | Arg | Gly | Tyr |
| Leu | Tyr | Ile | Ala 915 | Ile | Glu | Tyr | Ala 920 | Pro | Tyr | Gly | Asn 925 | Leu | Leu | Asp | Phe |
| Leu | Arg 930 | Lys | Ser | Arg | Val | Leu | Glu 935 | Thr | Asp | Pro | Ala 940 | Phe | Ala | Arg | Glu |
| His 945 | Gly | Thr | Ala | Ser | Thr 950 | Leu | Ser | Ser | Arg | Gln 955 | Leu | Leu | Arg | Phe | Ala 960 |

Ser Asp Ala Ala Asn Gly Met Gln Tyr Leu Ser Glu Lys Gln Phe Ile
 965 970 975
 His Arg Asp Leu Ala Ala Arg Asn Val Leu Val Gly Glu Asn Leu Ala
 980 985 990
 Ser Lys Ile Ala Asp Phe Gly Leu Ser Arg Gly Glu Glu Val Tyr Val
 995 1000 1005
 Lys Lys Thr Met Gly Arg Leu Pro Val Arg Trp Met Ala Ile Glu Ser
 1010 1015 1020
 Leu Asn Tyr Ser Val Tyr Thr Thr Lys Ser Asp Val Trp Ser Phe Gly
 1025 1030 1035 1040
 Val Leu Leu Trp Glu Ile Val Ser Leu Gly Gly Thr Pro Tyr Cys Gly
 1045 1050 1055
 Met Thr Cys Ala Glu Leu Tyr Glu Lys Leu Pro Gln Gly Tyr Arg Met
 1060 1065 1070
 Glu Gln Pro Arg Asn Cys Asp Asp Glu Val Tyr Glu Leu Met Arg Gln
 1075 1080 1085
 Cys Trp Arg Asp Arg Pro Tyr Glu Arg Pro Pro Phe Ala Gln Ile Ala
 1090 1095 1100
 Leu Gln Leu Gly Arg Met Leu Glu Ala Arg Lys Ala Tyr Val Asn Met
 1105 1110 1115 1120
 Ser Leu Phe Glu Asn Phe Thr Tyr Ala Gly Ile Asp Ala Thr Ala Glu
 1125 1130 1135
 Glu Ala

WHAT IS CLAIMED IS:

1. An isolated nucleotide sequence encoding a *tie-2* protein.
- 5 2. A cDNA nucleotide sequence encoding a *tie-2* protein.
- 10 3. A cDNA nucleotide sequence encoding a member of the MCK-10 family of proteins in which the nucleotide sequence encodes the amino acid sequence of FIG. 2 (SEQ. ID NO:), or which is capable of selectively hybridizing to the DNA sequence of FIG. 1 (SEQ. ID NO:).
- 15 4. A recombinant DNA vector containing a nucleotide sequence that encodes a *tie-2* protein.
- 20 5. A recombinant DNA vector containing a nucleotide sequence that encodes a *tie-2* fusion protein.
- 25 6. The recombinant DNA vector of Claim 4 in which the *tie-2* nucleotide sequence is operatively associated with a regulatory sequence that controls the *tie-2* gene expression in a host.
- 30 7. The recombinant DNA vector of Claim 5 in which the *tie-2* fusion protein nucleotide sequence is operatively associated with a regulatory sequence that controls the *tie-2* fusion protein gene expression in a host.
- 35 8. The DNA of Claim 2, 3, 4, 5, 6 or 7 in which the nucleotide sequence is capable of hybridizing under standard conditions, or which would be capable of hybridizing under standard conditions but for the

degeneracy of the genetic code to the DNA sequence of
FIG. 1.

9. An engineered host cell that contains the
recombinant DNA vector of Claims 4, 5, 6, or 7.

5 10. An engineered cell line that contains the
recombinant DNA expression vector of Claim 6 and
expresses *tie-2*.

10 11. An engineered cell line that contains the
recombinant DNA expression vector of Claim 7 and
expresses *tie-2* fusion protein.

15 12. The engineered cell line of Claim 10 which
expresses the *tie-2* on the surface of the cell.

13. The engineered cell line of Claim 11 that
expresses the *tie-2* fusion protein on the surface of
the cell.

20 14. A method for producing recombinant *tie-2*,
comprising:
(a) culturing a host cell transformed with the
recombinant DNA expression vector of Claim 9
and which expresses the *tie-2*; and
25 (b) recovering the *tie-2* gene product from the
cell culture.

15. A method for producing recombinant *tie-2*
fusion protein, comprising:
30 (a) culturing a host cell transformed with the
recombinant DNA expression vector of Claim
11 and which expresses the *tie-2* fusion
protein; and
35 (b) recovering the *tie-2* fusion protein from the
cell culture.

16. An isolated recombinant *tie-2* receptor protein.

17. A fusion protein comprising *tie-2* linked to a heterologous protein or peptide sequence.

5

18. An oligonucleotide which encodes an antisense sequence complementary to the *tie-2* nucleotide sequence, and which inhibits translation of the *tie-2* gene in a cell.

10

19. The oligonucleotide of Claim 18 which is complementary to a nucleotide sequence encoding the amino terminal region of the *tie-2*.

15

20. A monoclonal antibody which immunospecifically binds to an epitope of the *tie-2*.

20

21. The monoclonal antibody of Claim 20 which competitively inhibits the binding of ligand to the *tie-2*.

22. The monoclonal antibody of Claim 20 which is linked to a cytotoxic agent.

25

23. The monoclonal antibody of Claim 20 which is linked to a radioisotope.

24. A method for screening and identifying antagonists of *tie-2*, comprising:

30

(a) contacting a cell line that expresses *tie-2* with a test compound; and

(b) determining whether the test compound inhibits the binding of *tie-2* ligand and the cellular effects of ligand binding on the cell line,

35

in which antagonists are identified as those compounds that inhibit both the binding and cellular effects of *tie-2* ligand binding on the cell line.

25. A method for screening and identifying agonists of *tie-2* ligand, comprising:

- 5 (a) contacting a cell line that expresses the *tie-2* with a test compound in the presence and in the absence of ligand;
- (b) determining whether, in the presence of
10 ligand, the test compound inhibits the binding of ligand to the cell line and;
- (c) determining whether, in the absence of the ligand, the test compound mimics the cellular effects of ligand on the
15 cell line

in which agonists are identified as those test compounds that inhibit the binding but mimic the cellular effects of ligand on the cell line.

20 26. The method according to Claim 24 or 25 in which the cell line is a genetically engineered cell line.

25 27. The method according to Claim 24 or 25 in which the cell line endogenously expresses the *tie-2*.

28. A method for screening and identifying antagonists of *tie-2* activity comprising:

- 30 (a) contacting *tie-2* protein with a random peptide library such that *tie-2* will recognize and bind to one or more peptide species within the library;
- (b) isolating the *tie-2*/peptide combination;
- 35 (c) determining the sequence of the peptide isolated in step c; and

- (d) determining whether the test compound inhibits the biological activity of *tie-2*.

in which agonists are identified as those peptides that inhibit the binding but mimic the cellular effects of *tie-2*.

5

29. A method for screening and identifying agonists of *tie-2* comprising:

- (a) contacting *tie-2* protein with a random peptide library such that *tie-2* will recognize and bind to one or more peptide species within the library;
- (b) isolating the *tie-2*/peptide combination;
- (c) determining the sequence of the peptide isolated in step c; and
- (d) determining whether, in the absence of the *tie-2* ligand, the peptide mimics the cellular effects of *tie-2*.

10
15
20

30. The method according to Claim 28 or 29 in which the *tie-2* protein is genetically engineered.

31. A method of modulating the endogenous enzymatic activity of the tyrosine kinase *tie-2* receptor in a mammal comprising administering to the mammal an effective amount of a ligand to the *tie-2* receptor protein to modulate the enzymatic activity.

25

32. The method of Claim 31 in which the enzymatic activity of the receptor protein is decreased.

30

33. A recombinant vector containing a nucleotide sequence that encodes a truncated *tie-2* which has

35

dominant-negative activity which inhibits the biological activity *tie-2*.

34. The recombinant vector of claim 33 in which the vector is a retrovirus vector.

5

35. An engineered cell line that contains the recombinant DNA vector of Claim 34 and expresses truncated *tie-2*.

10

36. An engineered cell line that contains the recombinant vector of Claim 35 and produces infectious retrovirus particles expressing truncated *tie-2*.

15

37. An isolated recombinant truncated *tie-2* receptor protein which has dominant-negative activity which inhibits the biological activity of *tie-2*.

20

38. A method of modulating the biological activity of *tie-2* in a mammal comprising administering to the mammal an effective amount of truncated *tie-2* receptor protein which inhibits the biological activity of *tie-2* activation.

25

30

35

AGCAGGAGCC GGAGCAGGAG CAGAAGATAA GCCTTGGATG AAGGGCAAGA TGGATAGGCC TCGCTCTGCC 70
 CCAAGCCCTG CTGATACCAA GTGCCTTTAA GATACAGCCT TTCCCATCCT AATCTGCAAA GGAAACAGGA 140
 AAAAGGAACT TAACCCTCCC TGTGCTCAGA CAGAAATGAG ACTGTTACCG CCTGCTTCTG TGGTGTTCCT 210
 CCTTGCCGCC AACTTGTAAG CAAGAGCGAG TGGACCATGC GAGCGGGAAG TCGCAAAGTT GTGAGTTGTT 280
 GAAAGCTTCC CAGGGACTCA TGCTCATCTG TGGACGCTGG ATGGGGAGAT CTGGGGAAGT ATGGACTCTT 350
 TAGCCGGCTT AGTTCTCTGT GGAGTCAGCT TGCTCCTTTA TGGAGTAGTA GAAGGTGCCA TGGACCTGAT 420
 CTTGATCAAT TCCCTACCTC TTGTGTCTGA TGCCGAAACA TCCCTCACCT GCATTGCCTC TGGGTGGCAC 490
 CCCCATGAGC CCATCACCAT AGGAAGGGAC TTTGAAGCCT TAATGAACCA GCACCAAGAT CCACTGGAGG 560
 TTAACAAGA TGTGACCAGA GAATGGGCGA AAAAAGTTGT TTGAAGAGA GAAAAGGCCA GTAAGATTAA 630
 TGGTGCTTAT TTCTGTGAAG GTCGAGTTGG AGGACAGGCT ATAAGGATAC GGACCATGAA GATGCGTCAA 700
 CAAGCGTCCT TCCTACCTGC TACTTTAACT ATGACCGTGG ACAGGGGAGA TAATGTGAAC ATATCTTTCA 770
 AAAAGGTGTT AATTAAAGAA GAAGATGCAG TGATTIACAA AAATGGCTCC TTCATCCACT CAGTGCCCCG 840
 GCATGAAGTA CCTGATATTT TAGAAGTTCA CTTGCCGCAT GCTCAGCCCC AGGATGCTGG TGTGTACTCG 910
 GCCAGGTACA TAGGAGGAAA CCTGTTTACC TCAGCCTTCA CCAGGCTGAT TGTTGGGAGA TGTGAAGCTC 980
 AGAAGTGGGG GCCCGACTGT AGCCGTCTTT GTACTACTTG CAAGAACAAT GGAGTCTGCC ATGAAGATAC 1050
 CGGGGAATGC ATTTGCCCTC CTGGGTTTAT GGGGAGAACA TGTGAGAAAG CTTGTGAGCC GCACACATTT 1120
 GGCAGGACCT GTAAAGAAAG GTGTAGTGGG CCAGAAGGAT GCAAGTCTTA TGTGTTCTGT CTCCAGACC 1190
 CTTACGGGTG TTCTGTGCC ACAGGCTGGA GGGGTTGCA GTGCAATGAA GCATGCCCAT CTGGTTACTA 1260
 CGGACCAGAC TGTAAGCTCA GGTGCCACTG TACCAATGAA GAGATATGTG ATCGGTTCCA AGGATGCCTC 1330
 TGCTCTCAAG GATGGCAAGG GCTGCAGTGT GAGAAAGAAG GCAGGCCAAG GATGACTCCA CAGATAGAGG 1400
 ATTTGCCAGA TCACATTGAA GTAAACAGTG GAAAATTTAA CCCCATCTGC AAAGCCTCTG GGTGGCCACT 1470

FIG.1A

1/16

SUBSTITUTE SHEET (RULE 26)

ACCTACTAGT GAAGAAATGA CCCTAGTGAA GCCAGATGGG ACAGTGCTCC AACCAAATGA CTTCAACTAT 1540
ACAGATCGTT TCTCAGTGGC CATATTCCT GTCACCGAG TCTTACCTCC TGACTCAGGA GTCTGGGTCT 1610
GCAGTGTGAA CACAGTGGCT GGGATGGTGG AAAAGCCTTT CAACATTTCC GTCAAAGTTC TTCCAGAGCC 1680
CCTGCACGCC CCAAATGTGA TTGACACTGG ACATAACTTT GCTATCATCA ATATCAGCTC TGAGCCTTAC 1750
TTTGGGGATG GACCCATCAA ATCCAAGAAG CTTTTCTATA AACCTGTCAA TCAGGCCTGG AAATACATTG 1820
AAGTGACGAA TGAGATTTTC ACTCTCAACT ACTTGGAGCC GCGGACTGAC TACGAGCTGT GTGTGCAGCT 1890
GGCCCGTCCT GGAGAGGGTG GAGAAGGGCA TCCTGGGCCT GTGAGACGAT TTACAACAGC GTCTATCCGA 1960
CTCCCTCCTC CAAGAGGTCT CAGTCTCCTG CAAAAAGCC AGACAGCTCT AAATTTGACT TGGCAACCGA 2030
TATTACAAA CTCAGAAGAT GAATTTTATG TGGAAGTCGA GAGGCGATCC CTGCAAACAA CAAGTGATCA 2100
GCAGAACATC AAAGTGCCTG GGAACCTGAC CTCGGTGCTA CTGAGCAACT TAGTCCCCAG GGAGCAGTAC 2170
ACAGTCCGAG CTAGAGTCAA CACCAAGGCG CAGGGGGAGT GGAGTGAAGA ACTCAGGGCC TGGACCCTTA 2240
GTGACATTCT CCCTCCTCAA CCAGAAAACA TCAAGATCTC CAACATCACT GACTCCACAG CTATGGTTTC 2310
TTGGACAATA GTGGATGGCT ATTCGATTTT TTCCATCATC ATCCGGTATA AGGTTGAGG CAAAAATGAA 2380
GACCAGCACA TTGATGTGAA GATCAAGAAT GCTACCGTTA CTCAGTACCA GCTCAAGGGC CTAGAGCCAG 2450
AGACTACATA CCATGTGGAT ATTTTTCCTG AGAACAAAT AGGATCAAGC AACCCAGCCT TTTCTCATGA 2520
ACTGAGGACG CTTCACATT CCCAGCCTC TGCAGACCTC GGAGGGGGAA AGATGCTACT CATAGCCATC 2590
CTTGGGTCGG CTGGAATGAC TTGCATCACC GTGCTGTTGG CGTTTCTGAT TATGTTGCAA CTGAAGAGAG 2660
CAAATGTCCA AAGGAGAATG GCTCAGGCAT TCCAGAACGT GAGAGAAGAA CCAGCTGTGC AGTTTAACTC 2730
AGGAACTCTG GCCCTTAACA GGAAGGCCAA AAACAATCCG GATCCCACAA TTTATCCTGT GCTTGACTGG 2800
AATGACATCA AGTTTCAAGA CGTGATCGGA GAGGGCAACT TTGGCCAGGT TCTGAAGGCA CGCATCAAGA 2870
AGGATGGGTT ACGGATGGAT GCCCCATCA AGAGGATGAA AGAGTATGCC TCCAAAGATG ATCACAGGGA 2940
CTTCGCAGGA GAACTGGAGG TTCTTTGTAA ACTTGGACAC CATCCAAACA TCATCAATCT CTTGGGAGCA 3010

FIG.1B

TGTGAACACC GAGGCTATTT GTACCTAGCT ATTGAGTATG CCCC GCATGG AAACCTCCTG GACTTCCTGC 3080
 GTAAGAGCAG AGTGCTAGAG ACAGACCCTG CTTTGTCCAT CGCCAACAGT ACAGCTTCCA CACTGTCTC 3150
 CCAACAGCTT CTTTATTTTG CTGCAGATGT GGGCGGGG ATGGACTACT TGAGCCAGAA ACAGTTTATC 3220
 CACAGGGACC TGGCTGCCAG AAACATTTTA GTTGGTGAAA ACTACATAGC CAAAATAGCA GATTTTGGAT 3290
 TGTACGAGG TCAAGAAGTG TATGTGAAA AGACAATGGG AAGGCTCCCA GTGCGTTGGA TGGCAATCGA 3360
 ATCACTGAAC TATAGTGTCT ATACAACCAA CAGTGATGTC TGGTCCTATG GTGTATTGCT CTGGGAGATT 3430
 GTTAGCTTAG GAGGCACCCC CTA CTGCGG ATGACGTGCG CGGAGCTCTA TGAGAAGCTA CCCCAGGGCT 3500
 ACAGGCTGGA GAAGCCCCTG AACTGTGATG ATGAGGTGTA TGATCTAATG AGACAGTGCT GGAGGGAGAA 3570
 GCCTTATGAG AGACCATCAT TTGCCAGAT ATTGGTGTCC TTAAACAGGA TGCTGGAAGA ACGGAAGACA 3640
 TACGTGAACA CCACACTGTA TGAGAAGTTT ACCTATGCAG GAATTGACTG CTCTGCGGAA GAAGCAGCCT 3710
AGAGCAGAAC TCTTCATGTA CAACGGCCAT TTCTCCTCAC TGGCGGAGA GCGCCTTGAC ACCTGTACCA 3780
 AGCAAGCCAC CCACTGCCAA GAGATGTGAT ATATAAGTGT ATATATTGTG CTGTGTTTGG GACCCTCCTC 3850
 ATACAGCTCG TCGGATCTG CAGTGTGTTT TGA CTCTAAT GTGACTGTAT ATACTGCTCG GAGTAAGAAT 3920
 GTGCTAAGAT CAGAATGCCT GTTCGTGGTT TCATATAATA TATTTTCTA AAAGCATAGA TTGCACAGGA 3990
 AGGTATGAGT ACAAATACTG TAATGCATAA CTTGTTACTG TCCTAGATGT GTTTGATATT TTTCTTTAC 4060
 AACTGAATGC TATAAAAGTG TTTTGCTGTG TACACATAAG ATACTGTTG TTAATAAAG CATTCCCTTG 4130
 ACAGCACAGG AAGAAAAGCG AGGGAAATGT ATGGATTATA TTAAATGTGG GTTACTACAC AAGAGGCCGA 4200
 ACATTCCAAG TAGCAGAAGA GAGGTCTCT CAACTCTGCT CCTCACCTGC AGAAGCCAGT TTGTTTGGCC 4270
 ATGTGACAAT TGTCTGTGT TTTTATAGCA CCCAAATCAT TCTAAATAT GAACATCTAA AAACTTTGCT 4340
 AGGAGACTAA GAACCTTTGG AGAGATAGAT ATAAGTACCG TCAAAAAACA AAACGCGGG ACTTACATT 4410
 ATTTTCTATA GTAATCTGTT GTACATTTTA AGAAGTAAAA CTAGGAATTT AGGAGTGATG TGTGACATT 4480
 CTGACATGGA GTTACCATCC CCACATGTAT CACATACTGT CATATTCCCA CATGTATCAC ACATGTATTG 4550
 TAAATTTTG TAGTTTGTAT CACTGTGAA TTTACTGTTG ATGTGGTAGC CACCTGCTGC AATGGTTCT 4620
 CTTGTAGGTG AATAAATGTC

FIG.1C

3/16

SUBSTITUTE SHEET (RULE 26)

MDSL AGLVLC GVSLLLYGV EGAMDLILIN SLPLVSDAET SLTCIASGWH PHEPITIGRD 60
 FEALMNQHQD PLEVTDQVTR EWAKKVWKR EKASKINGAY FCEGRVRCQA IRIRTMKMRQ 120
 QASFLPATLT MTVDRCGNVN ISFKKVLKE EDAVIYKNGS FIHSVPRHEV PDILEVHLPH 180
 AQPDAGVYS ARYIGGNLFT SAFTRLIVRR ¹CEAQKWGPCD SRPCTTCKNN GVCHEDTGEC 240
²ICPPGFMGRT CEKACEPHTF GRTCKERCSCG PEGCKSYVFC LPDPYGCSCA TGWRGLQONE 300
³ACPSGYYGPD CKLRCHCTNE EICDRFQGCL CSQGWQGLQC EKEGRPRMTP QIEDLPDHIE 360
 VNSGKFNPIK KASGWPLPTS EEMTLVKPDG TVLQPNDFNY TDRFSVAIFT VNRVLPPDSG 420
 VWCSVNTVA GMVEKPFNIS ¹VKVLPEPLHA PNVIDTGHNF AINISSEPY FGDGPIKSKK 480
 LFYKPVNQAW KYIEVTNEIF TLNLEPRTD YELCVQLARP ²GEGGEGHPGP VRRFTTASIG 540
 LPPPRGLSLL PKSQTALNLT WQPIFTNSEDE FYVEVERRS LQTTSDQQNI KVPGNLTSVL 600
 LSNLVPREQY TVRARVNTKA QGEWSEELRA WTLSDILPPQ ³PENIKISNIT DSTAMVSWTI 660
 VDGYSISSII IRYKVQKNE DQHIDVKIKN ATVTQYQLKG LEPETTYHVD IFAENNIGSS 720
 NPAFSHELRT LPHSPASADL GGGKMLLIAI LGSAGMTCIT VLLAFLIMLQ LKRANVQRRM 780
 AQAFQNVREE PAVQFNSGTL ALNRKAKNNP DPTIYPVLDW NDIKFQDV ¹EGNFGQVLKA 840
 RIKKQGLRMD AAIKRMKEYA SKDDHRDFAG ELEVLCCKLGH HPNIINLLGA CEHRGYLYLA 900
 IEYAPHGNLL DFLRKSRLVE ¹TPAFATANS TASTL ²SSQQL LHFAADVARG MDYLSQKQFI 960
 HRDLAARNIL VGENYIAKIA DFGLSRGQEV YVKKTMGRLP VRMMATESLN YSVYTTNSDV 1020
 WSYGVLLWEI VSLGGTPYCG MTCAELYEKL PQGYRLEKPL NCDDEVYDLM ROCWREKPYE 1080
 PSFAQILVS LNRMLEERKT YVNTTLYEKF TYAGIDCSAE EAA

FIG.2A

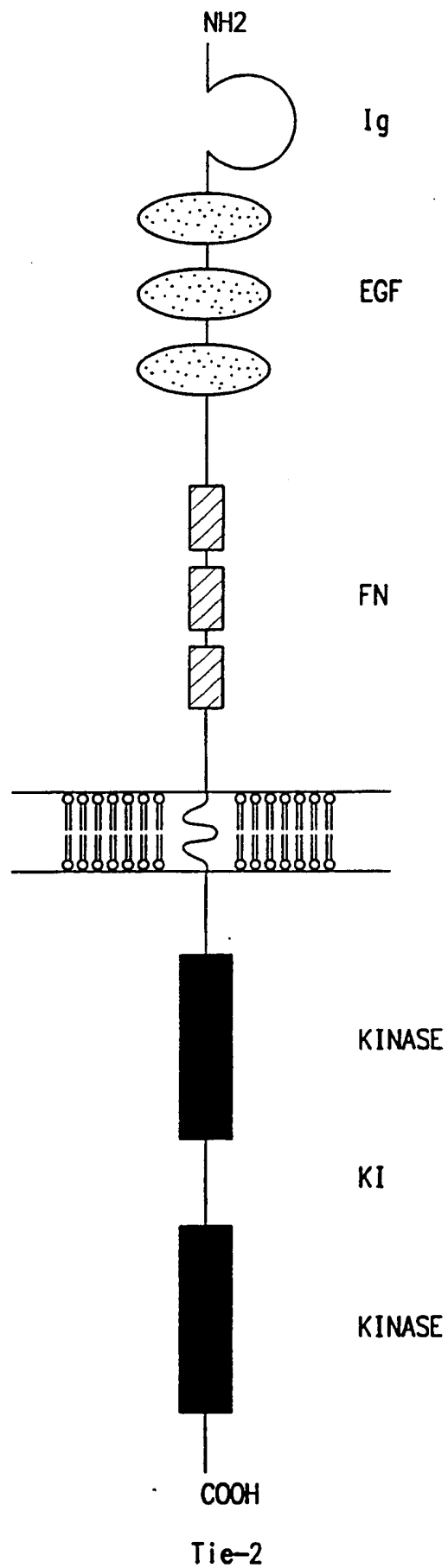


FIG.2B

1 100
lie-2 .MDSLGLVL CGVSLLLYGV VEGAMDIL I NSLPLVSDAE TSLTCLASGW HPHEPITIGR DFEALMNOHQ DPLEVTQDVT REWAKK..... VMKREKAS

TEK .---S---S-T---R---
lie MMWRVPPFL-..PI-F-ASH -GA-V-T-L AN-R-TDQR FF---V---E AGACRGSDAW GPPL-LEKDD RIVRTPGPP [RL-RNGSHQ VTLRGFS-P-

101 200
lie-2 KINGAYFCEG RVRGQAIRIR TMKMRQOASF LPATLIMTVD RGDNWNISFK KVL IKEEDAV IYKNGSF IHS VPRHEVPD. I LEVHLPHAOQ ODAGVYSARY

TEK ---E---K-VN---
lie DLV-VFS-V- GAGART-VI YVHNSPG-HL -DKV-H-N K-TAVL-AR VHKE-QT-VI WKS---YFYT LDW---AQ-GR FLLQ-NV- PSS-I---T-

201 300
lie-2 ICGNLTSAF TRLIVRRCEA QKWGPDCSR PCTCKKNGVC HEDTGECICP PGFMGRICEK ACEPHTFGRT CKERCSGPEG CKSYVFCCLPD PYGCSCATGW

TEK ---E-NHL-A-M---L---Q---
lie LEASPLG---F---G-G-GR -G-TKE -PG-LHG---DHD---V- -T-TR-Q -REGR-QS -Q-Q-PGIS- -RGLT---CS-

301 400
lie-2 RGLQCNEACP SCYGGPDCKL RCHCTNEEIC DRFGQCLCSQ GNGQLQCEKE GRPRMTPQIE DLPDHI EVNS CKFNPI.CKA SGWPLPTSEE MTLVKPDGTV

TEK K---H P-f---S-N-G-M---P---R- -I---K-V---N---
lie --S-Q---A P-HF-A-R- Q-Q-Q-CGT- -S-V-PS -H-VH---S D-...I---L NMASEL-f-L ETMPR-N-A- A-N-F-VRGS IE-R---

401 500
lie-2 LQPNDFNYTD RFSVAIFTN RVLPPDSGVW VCSWNTVAGM VEKPFNISVK VLPEPLHAPN VIDTGHNFAI INISSEPYFG DGPIKSKKLF YKPWN..QAW

TEK -H-K--H--H---IH -I---K-N---V---L---HYE---
lie -LSTKAIVEP EKTTEFE-P -LVLA---F- E-R-S-SG-Q DSRK-KVN- -P-V-A-R LL..KOSRQL WSPLVSFS- ---STVR-H -R-QDSTMD-

FIG.3A

SUBSTITUTE SHEET (RULE 26)

7/16

| | | |
|-------|--|------|
| 501 | 550 | 600 |
| tie-2 | KYIEV.TNEI FTILNYLEPRT DYELCVOLAR PCEGCEGHPG PVRRETTASI G.LPPRGLS LLPKSOTALN LTWOIFINS E..DEFYWEV ERRSLOTTSD | |
| TEK | OH-Q- V E V R N T PS D V-K- | |
| tie | ST-V-DPS-N V-MN-R-K-G-SVR S AW-PTLM-DCP EP-LQ-WLEG WHVEGTDR-R VS-SLPLVPG PLVGDGFLLR LWDGTRGQER | |
| 601 | 650 | 700 |
| tie-2 | QONIKVPGNL TSVLLSNLVP REQYTVRARV NTKAQEWSE ELRAWTLSDI LPPQENIKI SNITDSTAMV SWTIVDGY.. SISSIIIRYK VOGKNEDQHI | |
| TEK | N-H V D-T H-S-VI L T V | |
| tie | RE-VSS-QAR -A.-TG-T- GTH-QLDVQL YHCTLLGPAS PPAHVL-PPS G-A-RHLHA QALS-EIQL T-KHPEALPG P-KYWEVQ -A-GAG-P.L | |
| 701 | 750 | 800 |
| tie-2 | DVKIKNATVT QYQLKGLEPE TTYHVDIFA.ENN IGSSNPAFSH ELRTLPHSPA SADLGGKML LIAILGSAGM TCITVLLAFL IMLQLKRANV | |
| TEK | II A-Q V E-Q P L | |
| tie | WIDVDRPEE- STIIR-NAS -R-LFRMR-S IOGLGWS-T VEE-TLGNL QAECPVQESR A-EE-LDQQ- IL-W-VSA --L-I-A-L- TLVCIR-SCL | |
| 801 | 850 | 900 |
| tie-2 | QRRMAQAFON VR.EEPAVOF NSCTLALNRK AKNNPDPTIY PVLWDNDIKF QDVIGEGNFG QVLKARIKKD GLRMDAAIKR MKEYASKDDH RDFAGELEVL | |
| tek | | |
| TEK | V T | |
| tie | H-RTFTY-S GSG-TIL S T-T-R P-LQ-E-LS E-E-T- E-L IR-M K-N M L EN | |
| 901 | 950 | 1000 |
| tie-2 | CKLGHPNII NLLGACEHRG YLYLAIEYAP HGNLLDFLRK SRVLETDPAF AJANSTASTIL SSOQLLHFAA DVARGMDYLS QKQF IHRDLA ARNII LVCENY | |
| tek | M | |
| TEK | | |
| tie | KN I Y REHC R-R-S A-N-Q E V | |

FIG.3B

FIG. 3C

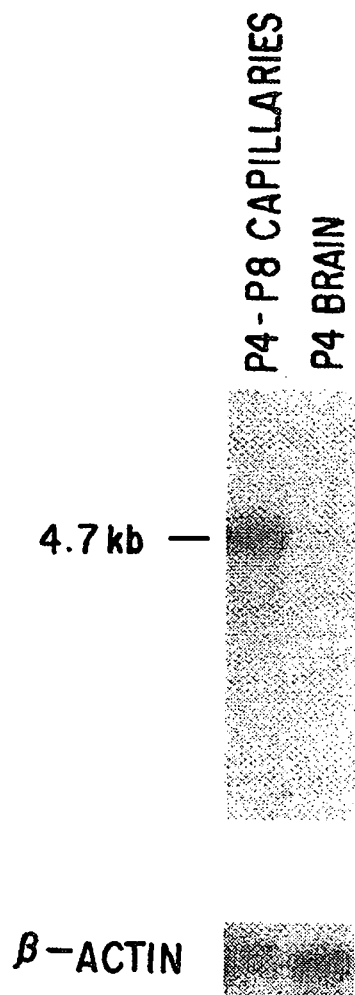


FIG. 4

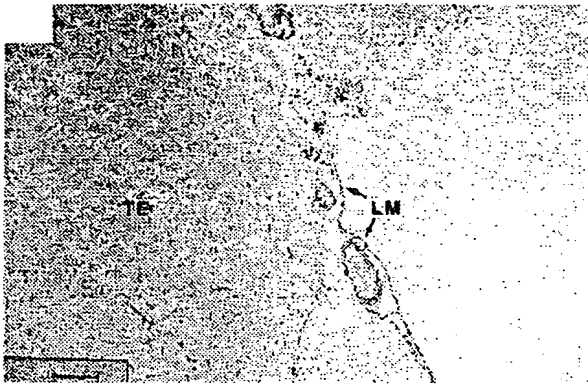


FIG.5A

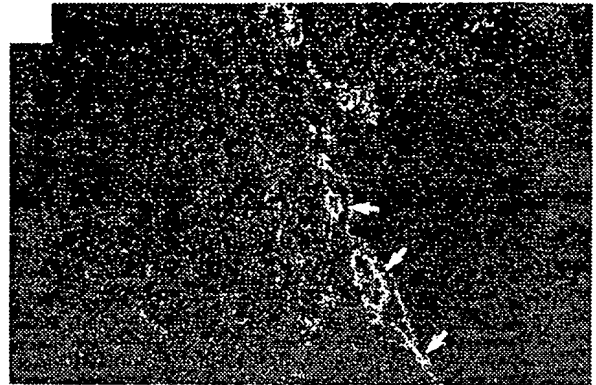


FIG.5B

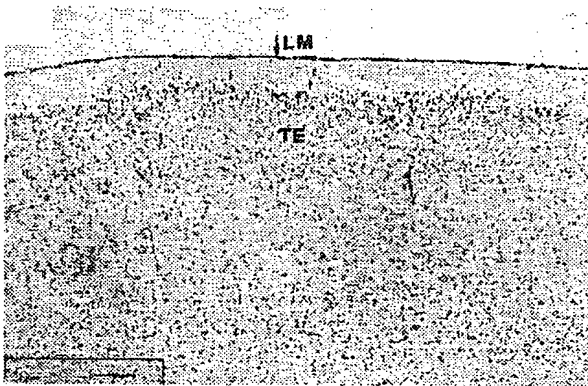


FIG.5C

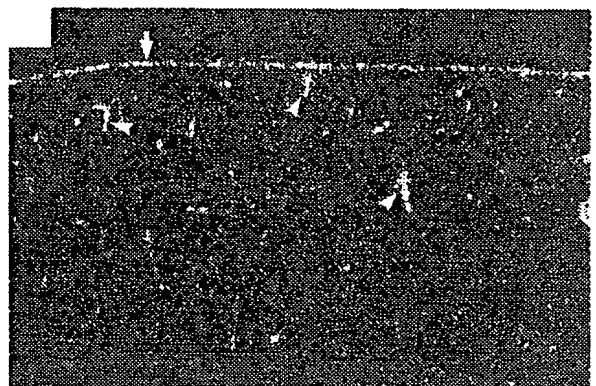


FIG.5D

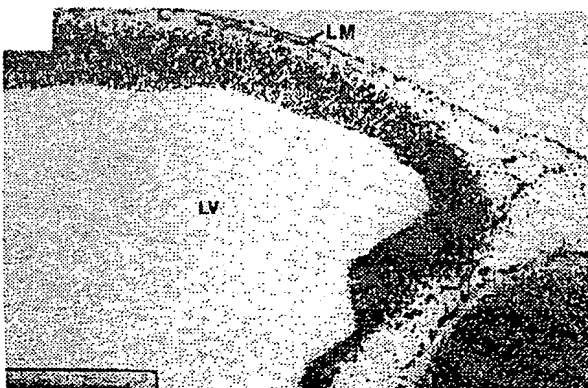


FIG.5E

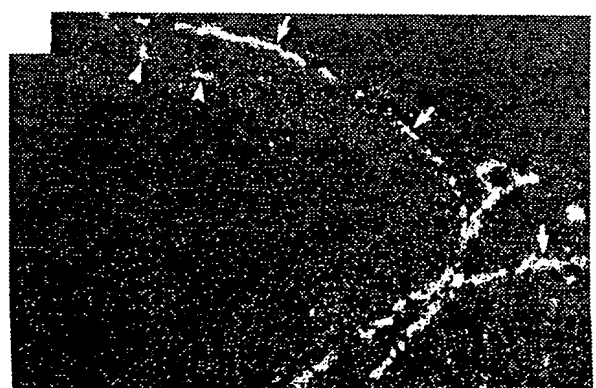


FIG.5F



FIG.5G



FIG.5H

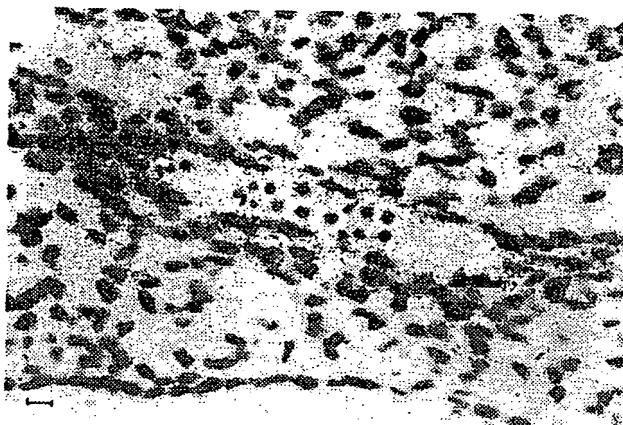


FIG.6A

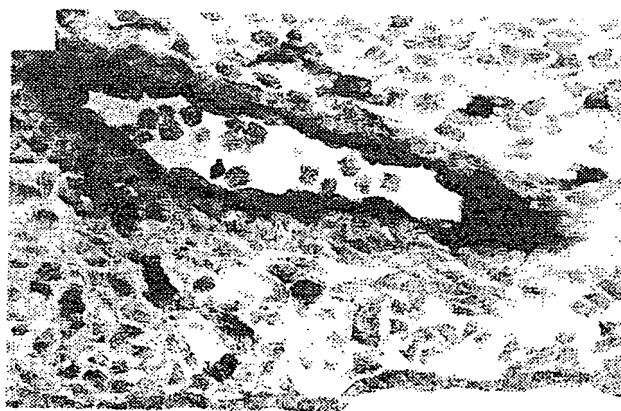


FIG.6B

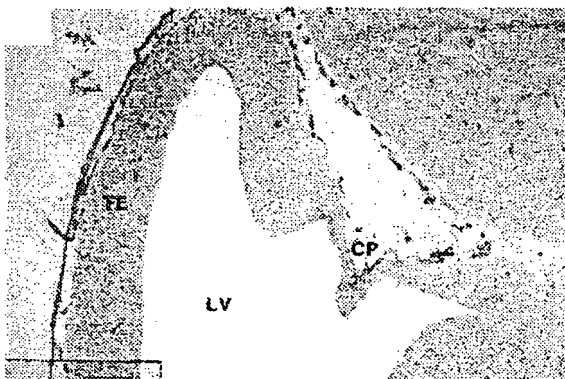


FIG. 7A

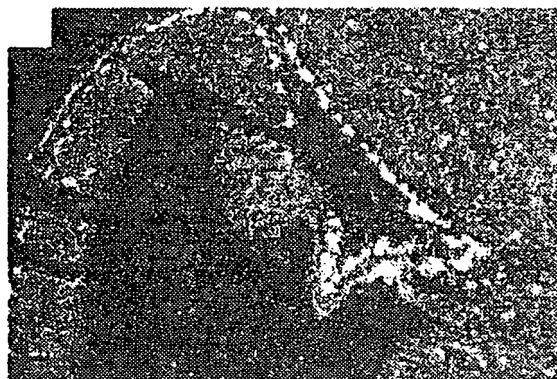


FIG. 7B

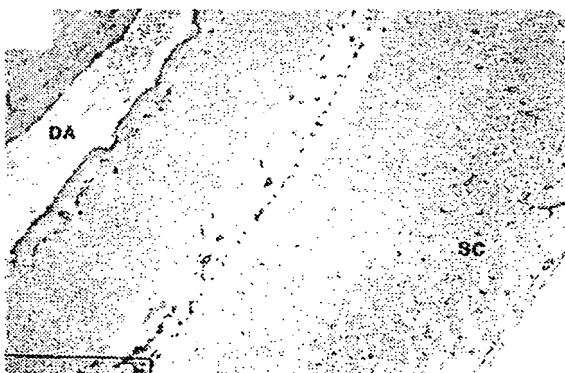


FIG. 7C

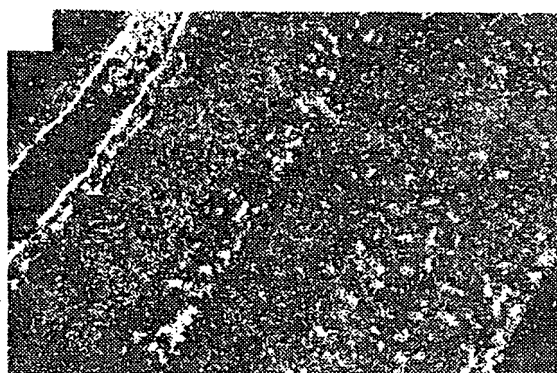


FIG. 7D

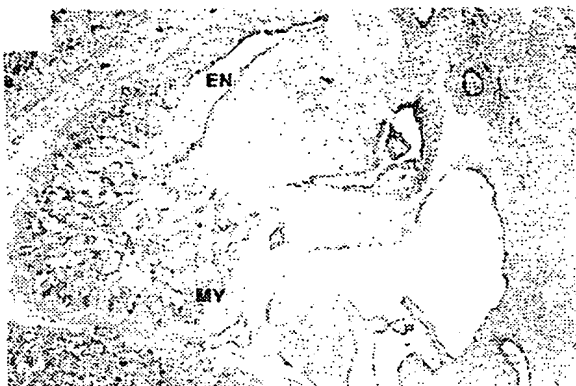


FIG. 7E



FIG. 7F

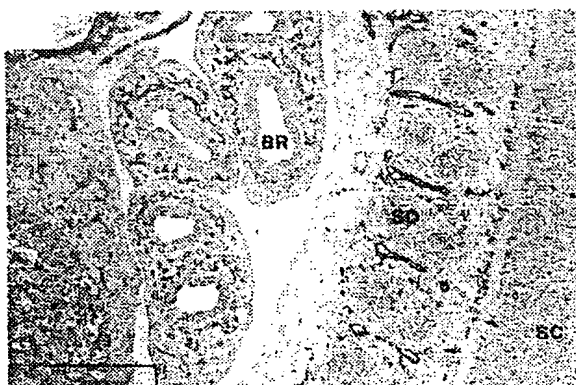


FIG. 7G

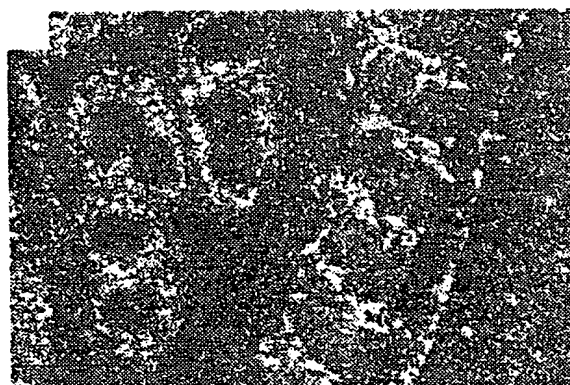


FIG. 7H

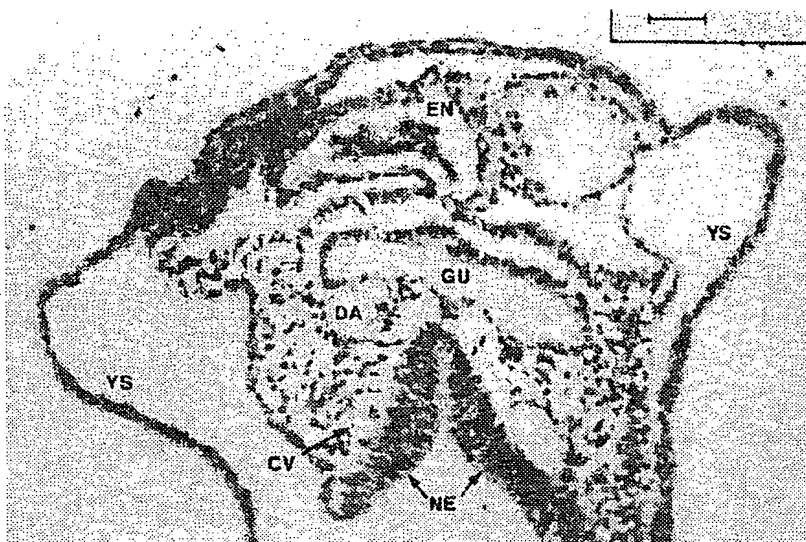


FIG.8A



FIG.8B

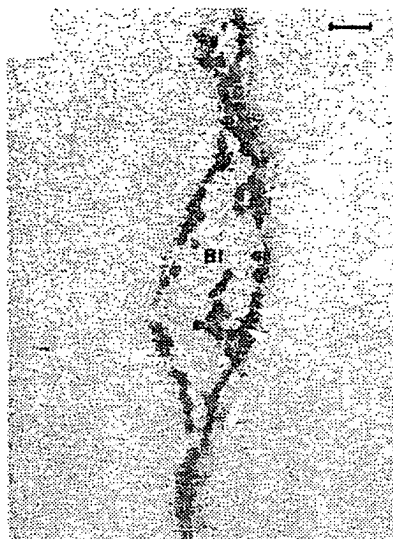


FIG.8C

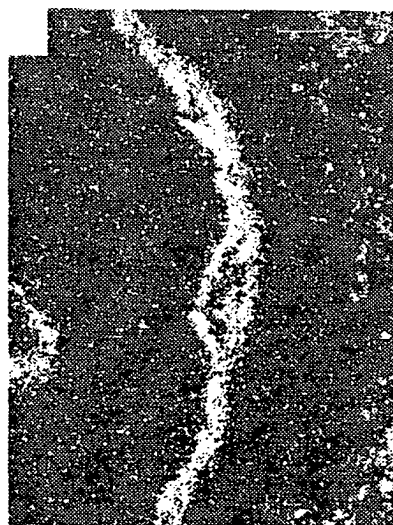


FIG.8D

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/63 C07K14/47 C07K14/515 C12N15/62 C12N5/16
C07K16/28

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|------------|--|-----------------------|
| X | DEVELOPMENT, vol. 119, 1993 pages 957-968, H. SCHNÜRCH ET AL. 'Expression of tie-2, a member of a novel family of receptor tyrosine kinases, in the endothelial cell lineage' *see the whole document* --- | 1-38 |
| X | GROWTH FACTORS, vol. 9, 1993 pages 99-105, A.S. RUNTING ET AL. 'tie-2, a putative protein tyrosine kinase from a new class of cell surface receptor' *see the whole document* --- -/-- | 1-38 |

☒ Further documents are listed in the continuation of box C.☐ Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

& document member of the same patent family

Date of the actual completion of the international search

2 March 1995

Date of mailing of the international search report

04.04.95

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentaan 2
NL - 2280 HV Rijswijk
Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl,
Fax (+ 31-70) 340-3016

Authorized officer

Marie, A

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|------------|---|-----------------------|
| X | ONCOGENE, vol. 8, 1993 pages 1631-1637, P.C. MAISONPIERRE ET AL. 'Distinct rat genes with related profiles of expression define a TIE receptor tyrosine kinase family' *see the whole document* --- | 1-38 |
| X | PNAS, vol. 90, 1993 pages 9355-9358, T.N. SATO ET AL. 'tie-1 and tie-2 define another class of putative receptor tyrosine kinase genes expressed in early embryonic vascular system' *see the whole document* ----- | 1-38 |